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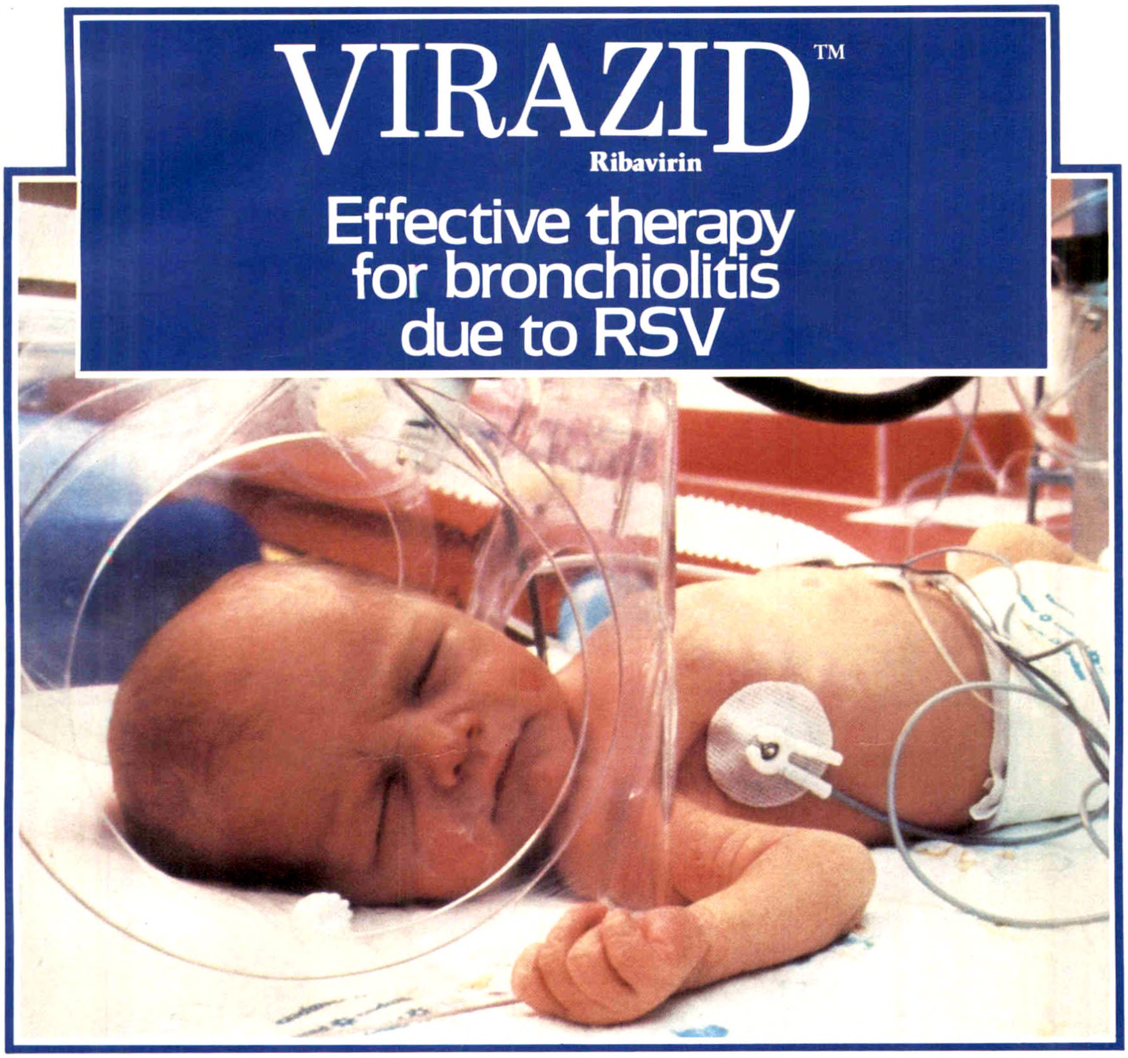

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Annotations

High frequency positive pressure ventilation in neonates

After a period of comparative stability, the clinician responsible for the care of the neonate in respiratory failure is faced with conflicting advice, not only on the settings he should be selecting on the ventilator,[1] but also whether he should be considering an entirely different form of respiratory support. The aim of this annotation is to look critically at the information available in an attempt to identify what represents a useful clinical advance and what should still be considered experimental.

The recommendations of Reynolds[2 3] that babies with respiratory distress syndrome should be ventilated at low respiratory rates (about 30/minute) and with a high inspiratory/expiratory ratio (1:1, 2:1) have been challenged for three main reasons[4]; firstly, the incidence of pneumothorax and bronchopulmonary dysplasia has remained high; secondly, the babies requiring respiratory support are now considerably less mature[5]; and thirdly, there are now numerous ventilators available on the market specifically designed for infants, which perform far better than those available when Reynolds was carrying out his classic studies. There have been three main approaches to these challenges, all coming under the umbrella term 'high frequency ventilation' but representing completely different techniques. These are: high frequency positive pressure ventilation, high frequency jet ventilation, and high frequency oscillation.

High frequency positive pressure ventilation

During the last 10 years new ventilators have been developed that function adequately at rates of up to 120/minute.[6] There is now increasing evidence to suggest that arterial oxygenation is at least as good at 60/minute as it is at 30/minute, and that the higher rate is associated with a lower incidence of pneumothorax.[7 8] Studies have also shown that active expiratory efforts generated by the baby against the inflation pressure (fighting the ventilator), a pattern that has been associated with a high incidence of pneumothorax,[9] may often be eliminated by raising the ventilator rate to 100 or even 120/minute.[10 11] This procedure is associated not only with an increase in carbon dioxide elimination, but often also with an improvement in arterial oxygenation, thereby avoiding the need for paralysis. There seems little benefit in raising the rate from 60–120/minute if the baby is apnoeic or is breathing synchronously with the ventilator at the lower rate.[12 13] It is, however, important to ensure that at these higher rates expiratory time is at least as long as inspiration, otherwise there will be insufficient time for expiration to be completed, leading to alveolar over distension as a result of positive end expiratory pressure.[1] This will not be apparent from measurements at the patient manifold, but may adversely effect cardiac output and, indeed, lead to pneumothoraces.

High frequency jet ventilation

This is the modification of a technique initially developed to provide respiratory support during bronchoscopy. Gas from a high pressure source is delivered in short bursts down a fine cannula, the tip of which is lying within the endotracheal tube and pointing down towards the periphery of the lung.[14] These 'bullets' of gas entrain additional gas down the endotracheal tube. For most studies rates of 100–200/minute have been selected, although the device has been used at rates as high as 400–600/minute. There have now been several studies showing that this is an effective form of ventilation,[14–16] which may lead to improvements in blood gas tensions when satisfactory results are not being obtained by conventional techniques. Initial hopes were that this form of ventilation would lead to less long term respiratory disability, as it is often possible to achieve adequate blood gas tensions with lower peak inflation pressures.[15] Unfortunately there has been a high incidence of damage to the trachea and large airways,[17] sometimes occurring within comparatively few hours of starting treatment. It was originally considered that the damage resulted from poor humidification[18]; it now seems to be caused by direct trauma from the high velocity bullets as well.[16 17] For this reason high frequency jet ventilation is best reserved for times when conventional ventilation has failed and when respiratory support of this type will only be required for a few hours—for example, in babies with diaphragmatic hernias who have yet to undergo operation. There may also be a place for this technique in babies with pulmonary interstitial emphysema who are proving difficult to ventilate conventionally.[19]

High frequency flow interruptors can best be classified as a subgroup of high frequency jet ventilators but differ in that the intermittent high pressure gas source is fed into a continuous positive airway pressure circuit immediately opposite the endotracheal tube connector. The pressure is released into the circuit either through a tube drilled across a rotating sphere mounted in a block[20] or across a shutter system. These devices seem to produce less large airway damage, can be used at frequencies of up to 20 Hz (1200/minute), and also lead to improvements in infants with pulmonary interstitial emphysema.[21]

High frequency oscillation

In this technique a sinusoidal volume generator feeds into a continuous positive airway pressure circuit close to the patient manifold. This produces a different wave form from the grossly distorted 'square wave' pattern produced by all the high frequency jet ventilators. The generator is either a piston, bellows, or a loud speaker driven by a synewave generator and audio amplifier. Much of the oscillating volume is lost into the continuous positive airway pressure circuit and the amount entering and leaving the endotracheal tube depends on a number of factors, including the comparative impedence of the baby's respiratory tract and the continuous positive airway pressure circuit, and the frequency selected. The high frequency oscillation may also be given at the same time as conventional ventilation. It has generally been accepted that this form of ventilation provides tidal exchange that is considerably less than the baby's anatomical dead space.[14] Experiments in animals and also in human adult volunteers have indicated that there are optimal frequencies at which the tidal exchange that is induced increases considerably.[14] These frequencies are thought to represent those that most closely match the natural resonance frequency of the respiratory system. A recent study has indicated[22] that even in the respiratory distress syndrome there is often a critical frequency (usually in the range of 12–24 Hz) at which even with oscillating pressures of less than 15 cm of water, tidal volumes in excess of anatomical dead space can be obtained.

All the studies to date have shown that high frequency oscillation improves elimination of carbon dioxide, possibly by direct alveolar ventilation and facilitated diffusion—that is, an exaggeration of the normal gas mixing process in the lung, and helped by oscillations between different parts of the lung.[23] The effects of high frequency oscillation on oxygenation have been less consistent.[14] It may be that this is determined more by the mean alveolar pressure and the presence of adequate carbon dioxide clearance than by improved delivery of oxygen to the terminal air sacks. It is certainly current practice to provide high continuous positive airway pressures, often in excess of 15 cm of water, during periods of high frequency oscillation. There is also evidence that high frequency oscillation can itself lead to over distension of the terminal air sacks as gas pushed into the air has insufficient time to escape. A recent study has shown that alveolar postive end expiratory pressure in excess of 4 cm of water is a common finding when the oscillating frequency exceeds 20 Hz (1200/minute).[24] Although alveolar positive end expiratory pressure may lead to increased oxygen uptake, there is evidence that high frequency oscillation can have adverse effects on cardiac output[25] and there is certainly a high incidence of pneumothorax associated with this form of ventilation. Perhaps the most worrying aspect is that the extent of the positive end expiratory pressure will be determined by the wave form produced by the device, the size of the endotracheal tube, whether there are any secretions present, the mechanical characteristics of the lungs and, probably, the extent to which the disease is homogeneous throughout the lungs. A recent study has shown that it is possible to measure the degree of alveolar positive end expiratory pressure produced by occluding the airway and measuring the back pressure,[26] providing there is an airtight seal between the endotracheal tube and the trachea. It may be that this measurement will be an essential part of ventilatory management when high frequency oscillation is used more extensively.

Conclusion

There are now a number of studies indicating that ventilatory rates of 60/minute with inspiratory times of less than 0·5 seconds produce good oxygenation, better carbon dioxide clearance, and have a lower incidence of pneumothorax than lower rates and inspiratory times of one second or greater. Babies who are fighting the ventilator at 60/minute can often be helped by increasing the respiratory rate in an attempt to produce a state in which the baby is breathing synchronously with the ventilator, taking care to ensure that expiration is at least as long as inspiration to prevent alveolar positive end expiratory pressure. High frequency jet ventilation is effective, but produces too many tracheal lesions to be acceptable for more than short term treatment. Although high frequency oscillation is an effective way of eliminating carbon dioxide, oxygenation is not always improved and there are potential hazards. It should still be considered as an experimental treatment that has not yet earned its place as a routine tool in clinical management.

References

[1] Ramsden CA, Reynolds EOR. Ventilator settings for newborn infants. Controversy and comment. *Arch Dis Child* 1987;**62:** 529–38.

[2] Reynolds EOR. Effects of alterations in mechanical ventilator settings on pulmonary gas exchange in hyaline membrane disease. *Arch Dis Child* 1971;**46:**152–9.

[3] Herman S, Reynolds EOR. Methods of improving oxygenation in infants mechanically ventilated for severe hyaline membrane disease. *Arch Dis Child* 1973;**48:**612–6.

[4] Greenough A, Milner AD. High frequency ventilation in the neonatal period. *Eur J Pediatr* 1987;**146:**446–9.

[5] Field DS, Milner AD, Hopkin IE, Madeley RJ. Changing patterns of neonatal respiratory distress. *Pediatr Pulmonol* 1987;**3:**231–5.

[6] Greenough A, Greenall F. Performance of respirators at fast rates commonly used in neonatal intensive care. *Pediatr Pulmonol* 1987;**3:**357–61.

[7] Heicher DA, Kasting DS, Richards JR. Prospective clinical comparison of two methods for mechanical ventilation of neonates: rapid rates and short inspiratory time versus slow rate and long inspiratory time. *J Pediatr* 1981;**98:**957–81.

[8] Pohlandt F, Bernsau V, Feilen KD, *et al.* Reduction of barotrauma in ventilated neonates by increase in ventilation frequency. First results of a prospective colaborative and randomised trial of two different ventilatory techniques. *Pediatr Res* 1985;**19:**1077.

[9] Greenough A, Wood S. Morely CJ, Davis JA. Pancuronium prevents pneumothoraces in ventilated premature babies who actively expire against positive pressure ventilation. *Lancet* 1984;**i:**1–4.

[10] Field DJ, Milner AD, Hopkin IE. Manipulation of ventilator settings to prevent active expiration against positive pressure inflation. *Arch Dis Child* 1985;**60:**1036–40.

[11] Greenough A, Morley CJ, Pool J. Fighting the ventilator—are fast rates an effective alternative to paralysis? *Early Hum Dev* 1986;**13:**189–94.

[12] Field DJ, Milner AD, Hopkin IE. High and conventional rates of positive pressure ventilation. *Arch Dis Child* 1984;**59:**115–8.

[13] Greenough A, Pool J, Greenall F, Morley C, Gamsu H. Comparison of different rates of artificial ventilation in preterm neonates with the respiratory distress syndrome. *Acta Paediatr Scand* 1987;**76:**706–12.

[14] Froese AB, Bryan AC. High frequency ventilation. *Am Rev Respir Dis* 1987;**135:**1363–74.

[15] Carlo WA, Chatburn RL, Martin RJ. Decrease in airway pressure during high frequency jet ventilation in infants with respiratory distress syndrome. *J Pediatr* 1984;**104:**101–7.

[16] Pokora T, Bing D, Mammel M. Neonatal high frequency jet ventilation. *Pediatrics* 1983;**72:**27–32.

[17] Mammel MC, Boros SJ. Airway damage and mechanical ventilation. *Pediatr Pulmonol* 1987;**3:**443–7.

[18] Metlay LA, MacPherson TA, Doshi N, Milley JR. A new iatrogenous lesion in newborns requiring assisted ventilation. *N Engl J Med* 1983;**309:**111–2.

[19] Harris TR, Chistensen RD. High frequency jet ventilation treatment of pneumonous interstitial emphysema. *Pediatr Res* 1984;**19:**3264.

[20] Frantz ID III, Werthammer J, Stark AR. High frequency ventilation in premature infants with lung disease: adequate gas exchange at low tracheal pressure. *Pediatrics* 1983;**71:**483–8.

[21] Gaylord MS, Quissell BH, Lair ME. High frequency ventilation in the treatment of infants weighing less than 1500 gms with pulmonary interstitial emphysema. *Pediatrics* 1987;**79:**915–21.

[22] Hoskyns EW, Milner AD, Hopkin IE. Oscillating volumes during ventilation with high frequency oscillation. *Early Hum Dev* 1988;**17:**96.

[23] Chang HK. Mechanisms of gas transport during ventilation by high frequency oscillation. *J Appl Physiol* 1984;**56:**553–63.

[24] Hoskyns EW, Milner AD, Hopkin IE. Dynamic lung inflation during high frequency oscillation in respiratory distress syndrome. *Early Hum Dev* 1987;**15:**186–7.

[25] DeLemos RA, Gerstmann DR, Clark RH, Guajardo A, Null DM. High frequency ventilation—the relationship between ventilator design and clinical strategy in the treatment of hyaline membrane disease and its complications: a brief review. *Pediatr Pulmonol* 1987;**3:**370–2.

[26] Bryan AC, Slutsky AS. Lung volume during high frequency ventilation. *Am Rev Respir Dis* 1986;**133:**928–30.

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Impact of AIDS on neonatal care

The emergence of HIV infection has forced health professionals to review their routine clinical practices. In the United Kingdom this has only recently become a problem in obstetrics and neonatal paediatrics as most HIV infection has been in adult men. Of the 8794 people identified as HIV antibody positive up to the end of June 1988, only 889 (10%) were female.[1] Most of these were intravenous drug abusers (54%), 25% acquired the infection through heterosexual contact and a much smaller number (7%) had been infected by transfusion of blood or its components. Clinical management of both the mother with proved HIV infection, or who is at high risk of infection, and her baby has become a matter of concern for all maternity departments as the number of infected women is increasing and experience from the United States suggests this trend will continue. In Massachusetts, screening all newborn infants early in 1987 showed that 2/1000 women giving birth must have been positive for HIV antibody, with a seropositive rate of 8/1000 in inner city hospitals.[2]

The Royal College of Obstetricians and Gynaecologists (RCOG) has published a report in conjunction with the British Paediatric Association and the Royal College of Midwives on 'Problems Associated with AIDS in relation to Obstetrics and Gynaecology', which also includes the care of the newborn infant.[3] The aim of the report is to produce guidelines for clinical practice. Many of the precautions it recommends apply specifically to mothers who are HIV seropositive or in high risk groups and their infants. Different management for high and low risk mothers and babies, a two tier approach, presupposes that women at high risk can be identified. With the voluntary antenatal HIV antibody screening currently practised many HIV positive mothers will not be identified antenatally,[4] and some infants who will subsequently be found to be HIV seropositive will be inappropriately managed as low risk.

Even if universal screening of all women antenatally were introduced, some would be missed because of false negative laboratory tests, women may be tested during the time lag between infection and seroconversion, and some women will refuse to be tested. In addition, as HIV infection becomes more prevalent, it is likely that the currently accepted 'high risk' categories will need to be broadened. In a specialty where emphasis is placed on the normality of pregnancy and in accommodating parents' wishes, it would seem retrogressive to divide all women into high and low risk groups and to modify their management accordingly.

HIV infection is mainly transmitted from mother to infant antenatally; the extent, if any, of transmission at birth and postnatally from breast milk is unclear. The precautions recommended are therefore made primarily for the protection of health workers and not for the baby.

The actual risk of infection to health care staff, through inoculation injuries and after exposure of broken skin and mucous membranes to the blood of infected patients, is extremely low with only 11 cases reported worldwide[5]; of these, only four were infected through inoculation injuries, although hundreds are known to have had penetrating wounds from needles or instruments used for HIV positive patients. There is good evidence, from studies of household contacts of AIDS patients, that HIV is not spread by close social contact nor is there evidence of faecal-oral, airborne, or salivary spread. It is therefore necessary to design infection control policies which, while protecting staff against the small but definite risk of infection, allows the patient to receive full medical care at all times.

Ideally, the same precautions should be applied to all mothers and babies, irrespective of their risk status. This also protects staff from other transmissible viral disorders, particularly hepatitis B, which is much more infectious than HIV. This single tier approach has to be balanced against the extra time and inconvenience for staff and expense. Some of the precautions, particularly masks and glasses, make the atmosphere more clinical and less friendly for parents. The acceptance and demand for more stringent precautions is also influenced by the prevalence of HIV in the community, which varies widely in different parts of the country.

Intrapartum care and delivery

At delivery, large amounts of blood, amniotic fluid, and lochia, all of which are potentially infective, are shed in an uncontrolled way. The RCOG report suggests that from now on, even at normal deliveries of low risk women, a gown should be worn over a plastic apron together with sterile gloves to avoid inadvertent and often unrecognised soiling of clothes. In addition it recommends that boots or shoes covered with plastic overshoes should be worn to prevent needle stick injuries to the feet and that all protective clothing and contaminated footwear be changed whenever staff leave the area.

Extra precautions are recommended during labour and delivery of HIV seropositive and high risk women. For intrapartum fetal monitoring, fetal scalp electrodes and fetal blood sampling should be avoided when possible as they result in a small open wound in a contaminated area and place the fetus at increased risk of infection. The delivery should be undertaken only by the minimum number of qualified doctors and midwives. At delivery, because of the variable and sometimes unexpected and explosive shedding of amniotic fluid, blood, and lochia, the report strongly recommends that all staff should wear full protective clothing with a gown over a plastic apron, surgical gloves, cap, mask, eye protection, and protective footwear.

In spite of the difficulties already described in identifying women at high risk and the undesirability of labelling and managing them differently the report considers that a two tier infection control policy is adequate for the United Kingdom at present. A particular problem in adopting a single policy for all women is the recommendation to avoid using fetal scalp electrodes and fetal blood samples. There is clearly an urgent need to develop non-invasive techniques for intrapartum monitoring.

Care of the newborn infant in the labour ward

When the mother is seropositive or in a high risk group the report advocates that the paediatrician performing neonatal resuscitation should also wear full protective clothing including gloves, a mask, and clear glasses for those not wearing spectacles. If a single tier code of practice is introduced this full protective clothing will be needed whenever resuscitation is performed. In emergencies, the minimum protection of gloves, mask, and eye protection should be used and need to be left readily to hand on the resuscitation table.

For all babies the report recommends that oral mucus extractors should no longer be used but replaced by mechanical suction or bulb syringes. The practice of aspirating meconium directly by oral suction through an endotracheal tube must also clearly be abandoned. This means that endotracheal tubes with a side vent attached directly to mechanical suction on the resuscitation table or a suction catheter passed down inside the endotracheal tube have to be used instead. Alternatively the suction catheter itself can be passed directly between the vocal cords which has the advantage that a wider bore catheter can be used, but is technically more difficult. To aspirate meconium immediately after delivery of the baby's head, mechanical suction apparatus that can only generate low pressures suitable for infants, needs to be used. The bulb syringes mentioned in the report are not currently available in Britain and modified oral mucus extractors are being developed and need to be evaluated. Bag and mask apparatus and laryngoscopes and other non-disposable items used for resuscitation will require sterilisation after use.

Cord blood sampling

Cord blood sampling is potentially hazardous because of the risk of blood spurting on the attendant and of needle stick injury. Cord sampling is desirable in the infants of seropositive and high risk women for testing for HIV antibody, antigen, and culture, having obtained maternal consent beforehand. The report suggests that instead of bleeding the umbilical cord directly into laboratory tubes a disposable funnel should be used to reduce contamination to the outside of the tubes. Unfortunately this technique is still messy and contamination with maternal blood is likely, making it unsuitable for many investigations. Whenever sampling is performed using a needle and syringe, clearly great care must be taken to avoid needle stick injury.

Washing of newborn infants

After clamping the cord, if the baby's condition allows, the report recommends that infants born to seropositive and high risk mothers should be washed with soap and water in the delivery room to remove any maternal blood and amniotic fluid. This recommendation is made as HIV virus has been found under experimental conditions to survive even in dried blood. Should this approach be adopted for all babies? Many mothers will doubtless be pleased to have their baby clean and not caked with dry blood for several days. What would be unwelcome would be the return of infants requiring admission to baby units because of hypothermia. So long as this can be avoided, it would be preferable for all babies to be bathed in the labour ward and this has been introduced successfully at St Mary's Hospital recently. Where conditions on the labour ward are unsuitable bathing can be done on admission to the postnatal ward.

Breast feeding

HIV appears to be present in high titre in cell free breast milk and in the cellular fraction of colostrum of HIV carriers.[6] This finding does not necessarily mean that breast milk is infectious, but there are four case reports where a breast fed infant is thought to have acquired HIV infection postnatally from mothers who received HIV seropositive blood in the postpartum period.[7-9] However, this may be an unrepresentative group as there may be a higher risk of viral transmission if a woman breast feeds around the time of acquisition of HIV, when there is a peak in antigen concentrations. Some breast fed babies of HIV positive mothers have escaped infection, and it has been suggested that the relative contribution of breast feeding to HIV infection is probably small compared with that of intrauterine transfusion.[10] A recent circular from the Department of Health recommends that to avoid possible added risk to the infant, all mothers known to be HIV infected should be discouraged from breast feeding.[11] With the evidence currently available, it appears prudent that women who are HIV positive should be counselled about possible risks and not encouraged to breast feed. The circular also extends this advice to include not only mothers known to be HIV infected but to all high risk women. As this includes women who have had sexual relations with people living in African countries (other than those on the Mediterranean) or those who have had sexual partners who have done so, this will apply to a significant proportion of women in many inner city areas As this recommendation is likely to affect adversely the current advocacy of breast feeding and the vast majority of these women will not be infected it has caused disquiet among many health care professionals.[12 13] Modification of this recommendation is expected in the near future. While these guidelines apply to the United Kingdom, in developing countries where bottle feeding may not be safely achieved, the risk from abandoning breast feeding needs to be weighed carefully against the potential risk of acquiring HIV infection.

Guidelines are also given in the Department of Health's letter regarding human milk banking. As with other donated tissues or organs, mothers wishing to donate milk should first answer a questionnaire to exclude those at high risk and should be known to be HIV antibody negative. The milk must be pasteurised and records maintained about the donors and recipients of the milk. Should a clinician wish to give unpasteurised donated human milk, the mother should be at low risk and antibody negative for HIV, the unpooled milk should be frozen and stored for three months after which the mother should be retested and, if negative, the specimen of milk can be used. The mother should continue to be HIV serotested monthly while she donates milk. The need to adhere to these guidelines is likely to result in an appreciable reduction in human milk banking and has led to the closure of some milk banks.

Postnatal care of the newborn infant

Once the baby of an HIV seropositive or high risk mother is washed free of blood he can be handled in the normal way, without gloves, and should be cared for in the same room as his mother. The report states, however, that gloves should be worn when performing any procedure involving blood, including blood sampling, administration of intramuscular injections, and when attending to the cord. All blood samples including Guthrie cards should be labelled 'biohazard' and placed in individual plastic bags. Bilirubin samples should not be centrifuged or estimated in a ward laboratory. Gloves should also be worn when passing nasogastric tubes or dealing with vomit or body fluids.

When babies require intensive care, it recommends the wearing of gloves whenever the baby is handled and full protective clothing including visors for invasive procedures.

If paediatricians are going to achieve a uniform approach in caring for all infants irrespective of their HIV status, the most important change in clinical practice surrounds the handling of blood. In contrast with adults, safe blood sampling is difficult in babies as their blood vessels are small and they do not hold still. Whenever a procedure is performed where blood may come into contact with the attendant's skin, surgical or latex gloves should be worn. Surgical gloves are expensive but should be used for invasive procedures as they are currently the only gloves with a British Standard Institution specification. Gloves made of vinyl or copolymer do not provide adequate protection for handling blood. Although many paediatricians dislike wearing gloves for intricate procedures, surgeons regularly perform delicate operations in gloves and reluctance among paediatricians should be overcome. Skin abrasions should be covered by closed unperforated waterproof plaster. Assistance is needed though when fixing cannulas in place as the adhesive tape sticks to the gloves. When arterial cannulation or any invasive procedures are performed, full protective clothing, including eye protection and surgical gloves, should be worn.

In intensive care, the RCOG report advocates that gloves should be worn whenever babies of HIV positive mothers are handled. The basis of this is that these babies have intravenous infusions which often need attention or resiting and are potential sources of blood escape. Should this be extended to cover all babies in intensive care units? Staff prefer not to have to put on gloves repeatedly as it is time consuming, handling babies is less pleasant, and gloves are expensive. An alternative is to wear gloves all the time rather than changing repeatedly. Such an approach has already been adopted by some health professionals—for example, dentists, and the gloved hands are washed between patients in the usual way. However, the washing of gloved hands is not recommended as detergents cause the gloves to deteriorate and there may be enhanced penetration of liquids through undetected holes.[14] A further disadvantage is that staff may be tempted to handle babies without prior handwashing, lulled into a false sense of security because they are wearing gloves. In addition, prolonged wearing of gloves is found by some to irritate the skin. Another approach is to advise staff to wear gloves only when they are taking or handling blood or if skin contact with blood is likely. Wearing a fresh pair of gloves for each procedure is clearly more hygienic and it also has the considerable advantage of a single policy for all babies whether in intensive or special care or on the postnatal wards irrespective of their mothers' HIV status. Further information on the advantages and disadvantages of these different approaches is required. Gloves do not provide protection against needle stick injury, and more attention will need to be paid to good technique to reduce this risk. Needles must not be resheathed but placed immediately into safe sharps disposable containers.

A further area of improvement required is in the handling of blood tests in ward laboratories. Clinicians performing these tests need to be trained and equipment maintained so as to conform with accepted laboratory standards of practice. A particular problem lies in safe bilirubin estimation in ward laboratories using blood samples taken into glass micropipettes. Some bilirubinometers use microcuvettes, requiring the glass pipette to be broken to transfer the plasma, a potentially hazardous procedure. Such practices must be modified.

No mention is made in the report of the expense of any of their recommendations. The increased use of protective clothing and gloves and alterations on ward laboratories are all going to involve increased expenditure, and priority should be given for adequate funds.

The field of HIV infection is constantly changing and guidelines will have to be regularly reassessed. The RCOG has already held a workshop on AIDS and obstetrics and gynaecology, the proceedings of which have been published,[15] and a second edition of the report will be produced. HIV infection has reminded us to respect blood as a potentially infectious fluid. In neonatal paediatrics we should try to avoid a two tier approach, with special policies for infants born to seropositive or high risk mothers. Instead, we must ensure that we provide routine clinical care of sufficiently high standard to be satisfactory for all infants, irrespective of their HIV status.

References

[1] PHLS Communicable Disease Surveillance Centre, 1988/27.

[2] Hoff R, Berard VP, Weiblen BJ, Mahoney-Trout L, Mitchell M, Grady GF. Seroprevalence of human immunodeficient virus among childbearing women. *N Engl J Med* 1988;**318:**525–30.

[3] *Report of the RCOG Sub-Committee on problems associated with AIDS in relation to obstetrics and gynaecology*. London: Royal College of Obstetricians and Gynaecologists, 1987.

[4] Krasinski K, Borkowsky W, Bebenroth D, Moore T. Failure of voluntary testing for HIV to identify infected parturient women in a high-risk population. *N Engl J Med* 1988;**318:**185.

[5] Expert Advisory Group on AIDS. AIDS: HIV–infected health care workers. London: HMSO, 1988.

[6] Thirty L, Sprecher-Goldberger S, Jonckheer T. Isolation of AIDS virus from cell-free breast milk of three healthy virus carriers. *Lancet* 1985;**ii:**891–2.

[7] Ziegler JB, Cooper DA, Johnson RO, Gold J. Postnatal transmission of AIDS-associated retrovirus from mother to infant. *Lancet* 1985;**i:**896–7.

[8] Lepage P, Van de Perre P, Carael M, *et al*. Postnatal transmission of HIV from mother to child. *Lancet* 1987;**ii:**400.

[9] Weinbreck P, Loustand V, Denis F, Vidal B, Mounier D, De Lumley L. Postnatal transmission of HIV infection. *Lancet* 1988;**i:**482.

[10] Senturia YD, Ades AE, Peckham CS, Giaquindo C. Breast-feeding and HIV infection. *Lancet* 1987;**ii:**400–1.

[11] Acheson D, Poole AAB. *HIV infection, breastfeeding and human milk banking*. London: DHSS, 1988.

[12] Logan S, Newell M-L, Ades T, Peckham CS. Breast-feeding and HIV infection. *Lancet* 1988;**i:**1346.

[13] Brierley J, Roth C, Warwick C. Breast-feeding and HIV infection. *Lancet* 1988;**i:**1346.

[14] Centers for Disease Control. Universal precautions for the prevention of transmission of HIV, HBV and other blood borne pathogens in health care settings. *MMWR* 1988;**37:**374–88.

[15] Royal College of Obstetricians and Gynaecologists. *Aids and obstetrics and gynaecology*. Proceedings of the Nineteenth Study Group. London: Royal College of Obstetricians and Gynaecologists, 1988.

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Effect of infusion rate of indomethacin on cerebrovascular responses in preterm neonates

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SUMMARY Cerebrovascular responses were studied in preterm infants by Doppler ultrasound and cerebral electrical impedance for one hour after intravenous indomethacin infusion for patent ductus arteriosus. Indomethacin in a dose of 0·2 mg/kg body weight was infused over five minutes in one group of infants (20 doses) and over 20 minutes in a second group of infants (16 doses). There were no significant differences between the two groups in birth weight (mean=1068 g, range 569–1950), gestational age (mean 28·4 weeks, range 25–31), or postnatal age (mean 18·1 days, range 6–42).

There was a significant reduction in both the Doppler mean flow velocity in the anterior cerebral artery (mean (SE) −20 (4·2)%) and peak amplitude of cerebral electrical impedance (−26 (3·9)%) within two minutes of starting the indomethacin infusion over five minutes. There was no significant change after the infusion over 20 minutes. There were no significant changes in blood pressure or carbon dioxide tensions after infusion at either rate. The results suggest that infusion of indomethacin over five minutes caused a potentially deleterious reduction in cerebral blood flow. No such reduction occurred when it was infused over 20 minutes.

Indomethacin is widely used to close haemodynamically important patent ductus arteriosus in neonates.[1 2] Its pharmacokinetics have been studied[3–6] and its comparative safety established in a collaborative study with a one year follow up.[7] Various dose regimens have been studied to maximise the clinical efficacy.[8 9]

Concern arose recently after the observation that therapeutic doses of indomethacin given to preterm neonates may cause a reduction in Doppler blood flow velocity in intracranial arteries.[10 11] Cowan[10] found a reduction of up to 75% in mean Doppler flow velocity in the intracranial arteries after four doses of 0·2 mg/kg body weight had been given in less than three minutes. Evans *et al*[11] found a mean reduction of 39% after 15 doses of 0·2 mg/kg body weight had been given within 15 seconds. Cerebral blood flow measured by the ^{133}xenon clearance technique has been reported to fall by a mean of 22% after six doses of 0·2 mg/kg body weight were given over one minute.[12]

Reduction in cerebral blood flow has also been reported in the fetuses and newborns of several animal species.[13–16] This may be caused by constriction of the microvasculature[17–19] in response to inhibition of prostaglandin synthesis within the endothelium.[20] Extrapolating from the result that the largest reduction in cerebral blood flow velocity occurred after the fastest infusion, Cowan[10] recommended that indomethacin be given slowly over at least five minutes.

This study was designed to investigate whether cerebrovascular responses measured by both Doppler ultrasound and cerebral electrical impedance were different when indomethacin was infused over five minutes from when it was infused over 20 minutes.

Patients and methods

The study was performed in two stages. The effect of indomethacin sodium trihydrate (Indocid PDA) infused over five minutes was studied after 20 doses in seven patients. In a further five patients the infusion period was changed to 20 minutes and the response after 16 doses was studied.

Each infant had clinical evidence of a haemodynamically important patent ductus arteriosus and they were all either receiving artificial ventilation or oxygen by headbox. All had received medical treatment consisting of fluid restriction and (in most cases) diuretics for at least 48 hours before the indomethacin was given. In 11 patients cross sectional echocardiography was done, and the presence of an important patent ductus arteriosus confirmed by a left atrial to aortic root diameter ratio of greater than 1·4:1. Clinical details of the infants are shown in the table.

Indomethacin was given intravenously in a dose of 0·2 mg/kg on three occasions 12 hours apart. Arterial or capillary carbon dioxide tensions were measured before the indomethacin was given. During 10 doses, transcutaneous carbon dioxide tensions were monitored continuously, as were heart rate and peripheral or umbilical arterial blood pressure during 20 doses.

Cerebral electrical impedance was measured as previously described.[21] The cardiac-synchronous pulsatile impedance change was digitised and 16 cardiac-synchronous wave forms averaged by a dedicated microprocessor. This signal reflected the changes in cranial blood volume during the cardiac cycle. The signal was recorded continuously by chart recorder, and the peak amplitude of cerebral electrical impedance measured. This measurement correlates in infants with the direction of change in cerebral blood flow estimated by ^{133}xenon clearance.[22]

Doppler ultrasound examination of blood flow velocity in the anterior cerebral artery was carried out through the anterior fontanelle with a continuous wave system using fast Fourier analysis of the Doppler frequency shift spectrum (Angioscan III). A 4 MHz probe was used in larger babies and an 8 MHz probe in smaller babies. The maximum signal was sought using sound and visual display of the spectrum from the region of the anterior cerebral artery. The Angioscan automatically calculated a mean frequency shift from the spectral information averaged over a memory block that varied from four to six heart beats, depending on the heart rate. The percentage change from the average of the three baseline measurements of the mean frequency shift was calculated. Doppler examinations were made intermittently, with the probe removed between examinations.

The repeatability of the two methods was calculated to ascertain whether any observed changes might be accounted for by poor repeatability of the techniques. The repeatability coefficient (defined as the limits within which 95% of the differences between two measurements of the same process lay)[23] was calculated from baseline measurements made one and three minutes before indomethacin was given, making certain that the clinical state of the baby was stable during this period.

Doppler examinations were carried out and peak amplitude of cerebral impedance noted at one, two, four, six, eight, and 10 minutes, and then at five minute intervals to one hour from the time indomethacin infusion was started.

Results are shown as mean (SE). Differences between the impedance and Doppler measurements before and after indomethacin were sought by two way analysis of variance, and if a significant difference existed the paired t test was used to establish the significance of differences between individual measurements. A p value of $<0{\cdot}05$ was accepted as significant. The influence of gestational and postnatal age on responses was assessed by multiple linear regression analysis.

Results

The responses of Doppler mean flow velocity and cerebral electrical impedance after infusion of indomethacin over five minutes are shown in figs 1 and 2. The maximum reduction in Doppler mean flow velocity (−20(4·2)%) occurred four minutes after starting the infusion, and the maximum reduction of cerebral electrical impedance (−26(3·9)%) occurred by one minute; these values were significantly different from the baseline values ($p<0{\cdot}01$ in both cases). The responses after indomethacin

Table *Comparability of the groups*

Five minute infusion			*20 Minute infusion*		
Birth weight (g)	*Gestational age (weeks)*	*Postnatal age (days)*	*Birth weight (g)*	*Gestational age (weeks)*	*Postnatal age (days)*
1340	29	6	1005	27	42
642	26	7	569	25	8
601	26	11	1123	27	8
1950	32	7	891	28	10
1100	31	21	1414	31	11
883	28	7, 26	1109	30	11
1259	29	8			

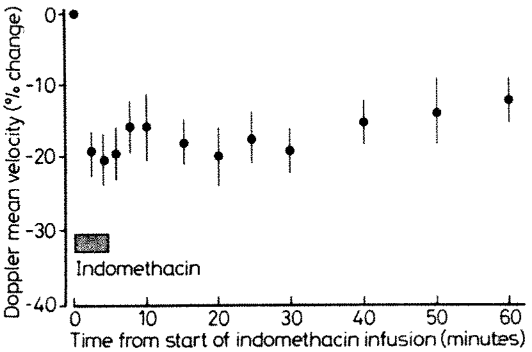

Fig 1 *Percentage change of Doppler mean flow velocity in the anterior cerebral artery after indomethacin infusion over five minutes. Figures are mean (SE).*

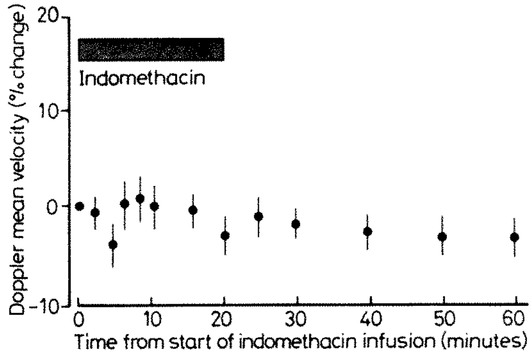

Fig 3 *Percentage change of Doppler mean flow velocity in the anterior cerebral artery after indomethacin infusion over 20 minutes. Figures are mean (SE).*

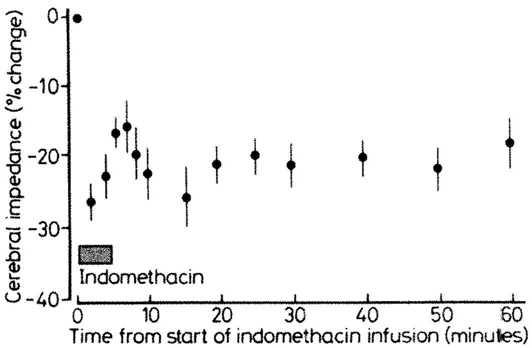

Fig 2 *Percentage change of cerebral electrical impedance peak amplitude after indomethacin infusion over five minutes. Figures are mean (SE).*

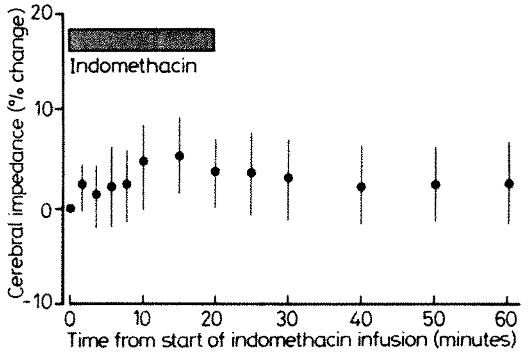

Fig 4 *Percentage change in cerebral electrical impedance peak amplitude after indomethacin infusion over 20 minutes. Figures are mean (SE).*

infusion over 20 minutes are shown in figs 3 and 4. There was a slight reduction (−4·2(2·3)%) in Doppler flow velocity and an increase (7·4(4·0)%) in cerebral electrical impedance, but these were not significantly different from the baseline values.

Responses to the first, second, and third doses of indomethacin infused over five minutes were not significantly different. The maximum changes in Doppler mean flow velocity for the three doses was −18(2·1)%, −22(4·1)%, and −20(5·4)%; for cerebral electrical impedance they were −20(6)%, −26(3·2)%, and −30(7·8)%, respectively.

There was no obvious trend in the continuous blood pressure recording after indomethacin given over either five or 20 minutes. Transcutaneous carbon dioxide tensions changed inconsistently and apparently independently of the dose of indomethacin. Of the 33 measurements made before the indomethacin was given, only two were more than 6 kPa (1 kPa=7·5 mm Hg). At these times, when carbon dioxide tensions were 7·1 and 7·8 kPa, the changes in Doppler mean flow velocity and cerebral electrical impedance were similar to the response in the same patient when carbon dioxide tension was <6 kPa. Multiple linear regression analysis of gestational age and postnatal age, with the mean maximum response of Doppler mean flow velocity and cerebral electrical impedance used sequentially as the dependent variable, showed no significant effect of either of these variables on response after indomethacin infused over five minutes.

The repeatability coefficient of Doppler mean flow velocity was 0·59 cm/s (mean 7·19 cm/s) and of cerebral electrical impedance was 2·4 mOhm (mean 22·2 mOhm). In 95% of cases therefore, assuming that the variable had not changed, repeated measurements could be expected to be within 16% of one another for Doppler mean flow velocity and within 22% for cerebral electrical impedance.

Discussion

The early reduction in Doppler mean flow velocity after the five minute infusion of indomethacin is similar to previously published results.[10,11] Cerebral blood flow decreases within two minutes in newborn animals.[17–19] This change is dose dependent, and in the newborn dog is about 20% with the dose used in this study,[14,24] which may be just enough to affect the cerebral circulation when infused rapidly but may not have an effect after slower infusion where instantaneous brain blood concentrations are lower. No previous studies have addressed the effect of different infusion rates on cerebrovascular responses. Another possible explanation as to why there was a difference in response after infusion at the two rates is that there was a difference in the two study groups. There were, however, no differences between the groups in gestational age, postnatal age, birth weight, carbon dioxide tension at the start of the infusion, and clinical state.

Whether there are any potentially deleterious effects of a reduction in cerebral blood flow associated with treatment with indomethacin is unknown. In experiments in animals, decreases in cerebral blood flow associated with indomethacin have not been associated with a reduction in cerebral metabolic rates of oxygen or glucose.[13,25,26]

The repeatability coefficients of both Doppler mean flow velocity and cerebral electrical impedance suggest that the reduction after the five minute infusion of indomethacin was not likely to have been accounted for by measurement variability. Results of both techniques suggest reductions in cerebral blood flow[27] of about the same magnitude as seen in newborn animals[15] and humans.[12]

Hypercapnia, postnatal age, and gestational age may affect the magnitude of the cerebrovascular response after infusion of indomethacin. Cerebral blood flow is reduced more when there is hypercapnia[26,28,29] but no similar effect of carbon dioxide tension was seen in this study on Doppler mean flow velocity or cerebral electrical impedance. In newborn dogs postnatal age is an important determinant of the magnitude of cerebrovascular response, but we saw no such effect. No studies have directly addressed the effect of gestational age on cerebrovascular responses but it seems likely that responses should be greater at lower gestational age down to an unknown lower limit.[15] We did not observe an effect of gestational age.

Indomethacin has effects on blood pressure control mediated by the autonomic nervous and renin angiotensin systems.[30,31] Evans *et al*[11] found a mean increase of 15% in blood pressure in the neonate after indomethacin had been given. In this study there was no consistent change in blood pressure after infusion at either rate.

The efficacy of indomethacin in closing the patent ductus arteriosus was not examined in this study, but it seems to be associated with steady state serum concentration.[3–6] Because the half life of indomethacin in the neonate is at least eight hours and usually about 20 hours[3,6] there should be no difference in serum concentrations after infusion at the different rates used in this study, and hence efficacy would be expected to be the same.

We recommend that in clinical practice and in studies to determine optimal dosage schedules indomethacin be infused over 20 minutes or longer. Until further data are available there must be concern about the possible deleterious effects of the apparent reduction in cerebral blood flow observed with shorter infusion periods.

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References

[1] Silove ED. Pharmacological manipulation of the ductus arteriosus. *Arch Dis Child* 1986;**61**:827–9.

[2] Goldberg SJ. Response of the patent ductus arteriosus to indomethacin treatment. *Am J Dis Child* 1987;**141**:250.

[3] Yaffe SJ, Friedman WF, Rogers D, Lang P, Ragni M, Saccar C. The disposition of indomethacin in preterm babies. *J Pediatr* 1980;**97**:1001–6.

[4] Brash AR, Hickey DE, Graham TP, Stahlman MT, Oates JA, Cotton RB. Pharmacokinetics of indomethacin in the neonate. *N Engl J Med* 1981;**305**:67–72.

[5] Yeh TF, Luken J, Raval D, Thalji A, Carr I, Pildes RS. Indomethacin treatment in small versus large premature infants with ductus arteriosus. *Br Heart J* 1983;**50**:27–30.

[6] Seyberth HW, Knapp G, Wolf D, Ulmer HE. Introduction of plasma indomethacin level monitoring and evaluation of an effective threshold level in very low birth weight infants with symptomatic patent ductus arteriosus. *Eur J Pediatr* 1983;**141**:71–6.

[7] Peckham GJ, Miettinen OS, Ellison RC, *et al.* Clinical course to one year of age in premature infants with patent ductus arteriosus: results of a multicentre trial of indomethacin. *J Pediatr* 1984;**105**:285–91.

[8] Mahony L, Caldwell RL, Girod DA, *et al.* Indomethacin therapy on the first day of life in infants with very low birth weight. *J Pediatr* 1985;**106**:801–5.

[9] Rennie JM, Doyle J, Cooke RW. Early administration of indomethacin to preterm infants. *Arch Dis Child* 1986;**61**:233–8.

[10] Cowan F. Indomethacin, patent ductus arteriosus, and cerebral blood flow. *J Pediatr* 1986;**109**:341–4.

[11] Evans DH, Levene MI, Archer LNJ. The effect of indomethacin on cerebral blood flow velocity in premature infants. *Dev Med Child Neurol* 1987;**29**:776–82.

[12] Pryds O, Greisen G, Johansen KH. Indomethacin and cerebral blood flow in premature infants treated for patent ductus arteriosus. *Eur J Pediatr* 1988;**147**:315–6.

[13] Hohimer AR, Richardson BS, Bissonette JM, Machida CM. The effect of indomethacin on breathing movements and cerebral blood flow and metabolism in the fetal sheep. *J Dev Physiol* 1985;**7**:217–28.

[14] Leffler CW, Busija DW. Arachidonate metabolism on the cerebral surface of newborn pigs. *Prostaglandins* 1985;**30**:811·8.

[15] Leffler CW, Busija DW. Arachidonic acid metabolites and perinatal cerebral hemodynamics. *Semin Perinatol* 1987;**11**: 31–42.

[16] Ment LR, Stewart WB, Duncan CC, Scott DT, Lambrecht R. Beagle puppy model of intraventricular hemorrhage: effect of indomethacin on cerebral blood flow. *J Neurosurg* 1983;**58**: 857–62.

[17] Pickard JD, Tamura A, Stewart M, *et al*. Prostacyclin, indomethacin and the cerebral circulation. *Brain Res* 1980;**197**:425–31.

[18] Dahlgren N, Siesjo BK. Effects of indomethacin on cerebral blood flow and oxygen consumption in barbiturate-anaesthetized normocapnic and hypercapnic rats. *J Cereb Blood Flow Metab* 1981;**1**:109–15.

[19] Crockard HA, Iannotti F, Ladds G. Cerebrovascular effects of prostaglandin inhibitors in the gerbil. *J Cereb Blood Flow Metab* 1982;**2**:67–72.

[20] Hagen AA, White RP, Robertson JT. Synthesis of prostaglandins and thromboxane B_2 by cerebral arteries. *Stroke* 1979;**10**:306–9.

[21] Colditz P, Pryds O, Greisen G, Murphy D, Rolfe P, Wilkinson AR. Cerebral electrical impedance: do indices derived from it provide information on cerebral blood flow in the neonate? *Scand J Clin Lab Invest* (in press).

[22] Colditz P, Greisen G, Pryds O. Comparison of electrical impedance and ^{133}xenon clearance for the assessment of cerebral blood flow in the newborn infant. *Pediatr Res* (in press).

[23] Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;**1**:307–10.

[24] Leffler CW, Busija DW, Beasley DG. Effect of therapeutic dose of indomethacin on the cerebral circulation of newborn pigs. *Pediatr Res* 1987;**21**:188–92.

[25] McCulloch J, Kelly PAT, Grome JJ, Pickard JD. Local cerebral circulatory and metabolic effects of indomethacin. *Am J Physiol* 1982;**243**:H416–23.

[26] Eriksson S, Hagenfeldt L, Law D, Patrono C, Pinca E, Wennmalm A. Effect of prostaglandin synthesis inhibitors on basal and carbon dioxide stimulated cerebral blood flow in man. *Acta Physiol Scand* 1983;**117**:203–11.

[27] Greisen G, Johansen K, Ellison PH, Fredricksen PS, Mali J, Friis-Hansen B. Cerebral blood flow in the newborn infant: Comparison of Doppler ultrasound and 133-xenon clearance. *J Pediatr* 1984;**104**:411–7.

[28] Sakabe T, Siesjo BK. The effect of indomethacin on the blood flow-metabolism couple in the brain under normal, hypercapnic and hypoxic conditions. *Acta Physiol Scand* 1979;**107**:283–4.

[29] Busija DW, Heistad DD. Effects of indomethacin on cerebral blood flow during hypercapnia in cats. *Am J Physiol* 1983;**244**:H519–H524.

[30] Anderson RJ, Berl T, McDonald KM, Schrier RW. Prostaglandins: effects on blood pressure, renal blood flow, sodium and water excretion. *Kidney Int* 1976;**10**:205–15.

[31] Dusting GJ, Moncada S, Vane JR. Prostaglandins, their intermediates and precursors: cardiovascular actions, and regulatory roles in normal and abnormal circulatory systems. *Prog Cardiovasc Dis* 1979;**21**:405–30.

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Acute effects of instillation of surfactant in severe respiratory distress syndrome

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SUMMARY Doppler ultrasound measurements of pulmonary blood flow in 20 babies with severe respiratory distress syndrome treated in a randomised controlled trial of surfactant replacement showed that the immediate improvement of oxygenation was not associated with a significant increase in pulmonary blood flow. Reduction in ventilator settings and increases in the extent of chest wall movements measured by a cardiorespiratory monitor suggested that the improvement after surfactant had been given was a result of alveolar stabilisation and increased pulmonary compliance. Further simultaneous studies of pulmonary blood flow and pulmonary compliance are needed to confirm these findings.

Natural surfactant has been used to reverse the effects of respiratory failure in animals.[1-5] These studies showed an almost instantaneous increase in arterial oxygen tension followed by a more gradual change in pH and arterial carbon dioxide tension. This rapid improvement in oxygenation was reminiscent of the action of tolazoline in babies with pulmonary hypertension, and we wondered if surfactant acted like a pulmonary vasodilator drug.

In lambs changes in pulmonary compliance can be detected 15–30 minutes after giving surfactant[1-3] but pulmonary blood flow either does not change[4] or increases after a delay of about two to three hours.[5] In immature baboons both compliance and pulmonary blood flow increase after instillation of surfactant, pulmonary blood flow by threefold.[6] As far as we are aware there have been no studies of pulmonary blood flow changes in babies after surfactant treatment.

Patients and methods

Based on the threefold increase in pulmonary blood flow in immature baboons after surfactant treatment[6] we estimated that to detect a similar change in babies with a power of 80% and a probability of <0.05 (two tailed test) would require a sample of 20 patients.

Serial measurements of pulmonary blood flow were made in 20 consecutive babies who were taking part in a randomised controlled trial of surfactant replacement.[7] All babies entering the study had severe respiratory distress syndrome requiring mechanical ventilation with >60% oxygen when less than 15 hours old.[7]

The surfactant was isolated from porcine lungs by chloroform-methanol extraction and further purified by liquid-gel chromatography[8]; it was given in a total dose of 200 mg/kg (2·5 ml/kg) through the endotracheal tube. Treated babies were disconnected from the ventilator and 1·25 ml/kg of surfactant was instilled into each main bronchus through a sterile feeding tube. After each instillation the baby was manually ventilated at a rate 40–60/minute for one minute using the same oxygen concentration and pressure of <35 cm H_2O before being reconnected to the ventilator without altering its settings. In the babies who acted as controls, manual ventilation without instillation of liquid was carried out. After enrolment into the study blood gases were measured at five, 15, 30, and 60 minutes, and appropriate changes in the ventilator settings were made to keep arterial oxygen tension about 7 kPa, arterial carbon dioxide tension about 6 kPa and pH greater than 7·3. The protocol required that the inspired oxygen concentration should be lowered first, and then the inspiratory pressure, the frequency, and the inspiratory: expiratory time ratio.[7]

Pulmonary blood flow was measured 15 minutes, one and four hours after instillation of surfactant with an ATL Mk V Duplex scanner. Pulmonary blood flow is difficult to measure directly but is equivalent to pulmonary venous return, which can be measured as flow across the aortic valve if there are no intracardiac shunts.[9] Such intracardiac shunts were excluded in all study babies by careful cross sectional and pulsed Doppler echocardiographic examinations.

Alveolar oxygen tension was calculated as previously described using the equation:[7] alveolar oxygen tension=fraction of inspired oxygen×94−arterial carbon dioxide tension/0·8 kPa. The ratio of arterial:alveolar oxygen tension was taken as a measure of oxygenation as it is not influenced by the amount of inspired oxygen. We used the product of mean airway pressure and ventilator rate as a measure of ventilation. If arterial carbon dioxide tension remains constant and spontaneous respirations are largely absent, the product of mean arterial pressure×ventilator rate should be a measure of ventilation that correlates inversely with compliance.[10]

Respiration traces were obtained from a Corometrics 512 monitor before, during, and after surfactant replacement. This monitor measures the extent of chest wall movement by recording changes in transthoracic impedance, which correlate indirectly with pulmonary compliance.[11]

The significance of differences between the groups was assessed by the Student's *t* test. In addition, 95% confidence intervals were calculated for the differences in mean values between treated and control babies.[12]

Results

The 12 treated babies had a mean (SD) birth weight of 1335 (312) g and gestational age 28·4 (1·1) weeks. The median age at enrolment was 5 hours (range 2–13). The eight control infants had a mean birth weight of 1401 (258) g and gestational age 28·8 (1·9) weeks; their median age at enrolment was 7 hours (range 2–15). Fifteen babies were given muscle relaxants during ventilation (eight treated and seven controls).

Fig 1 compares the changes in arterial to alveolar oxygen tension ratio with the calculated measure of mechanical ventilation and pulmonary blood flow in ml/kg/minute. There was an immediate increase in arterial:alveolar oxygen tension ratio so that treated babies had significantly higher ratios from 15 minutes to four hours (fig 1 and table). The table shows positive values for 95% confidence intervals of the difference between treated and control babies at 15 minutes and one hour. The product of mean arterial pressure×ventilator rate was significantly lower for treated babies by 15 minutes and remained so for the four hour period (fig 1) but wide confidence intervals permit only the four hour difference to be confidently accepted as applying to a larger population of babies (table). Pulmonary blood flow was not significantly different between treated and control infants and did not change after treatment with surfactant. 95% Confidence intervals of the difference between treated and control babies are wide for this measurement (table). Arterial carbon dioxide tensions and pH were similar in each group and did not change significantly after instillation of surfactant.

Fig 2 shows an example of a respiratory trace from the Corometrics 512 monitor of a baby before,

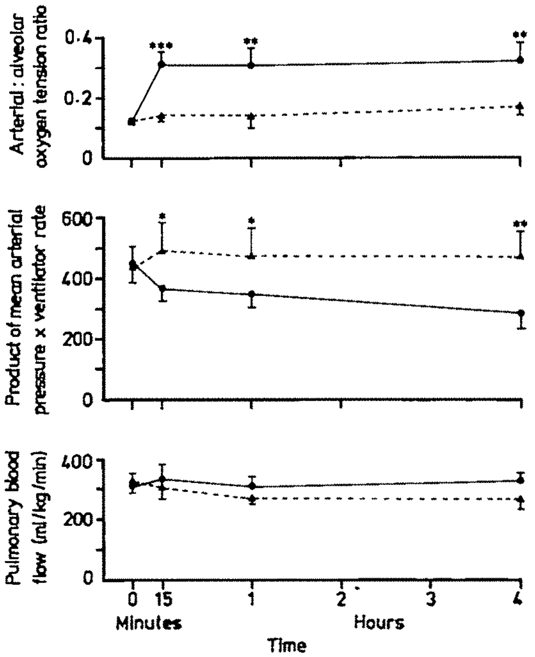

Fig 1 *Comparison of arterial:alveolar oxygen tension ratio, product of mean airway pressure×ventilator rate, and pulmonary blood flow for treated (●——●) and control (▲- - - -▲) babies after treatment with surfactant. Time 0 indicates values just before instillation of surfactant. Mean (SE), *p<0·05, **p<0·01, ***p<0·001.*

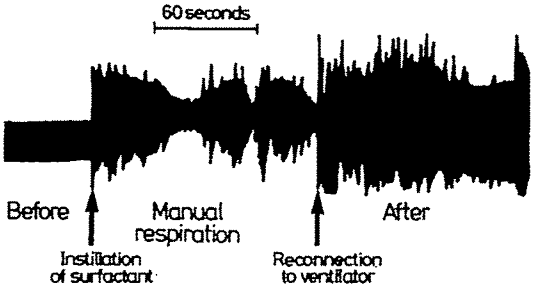

Fig 2 *Respiratory trace from Corometrics 512 monitor before, during, and after instillation of surfactant in a baby with severe respiratory distress syndrome.*

during, and after replacement of surfactant. The extent of chest wall movement (transthoracic impedance) increased immediately after surfactant had been given, and was maintained after the baby was reconnected to the ventilator. This pattern was seen in nearly all the treated babies, but similar changes were not seen in control babies.

Discussion

Natural surfactants have been shown to improve oxygenation in animals[1-5] and in babies.[7 8] The rise in arterial oxygen tension occurs immediately and precedes the decrease in arterial carbon dioxide tension and the increase in compliance.[3] Jacobs *et al* found that the surface tension of alveolar washings correlated better with arterial oxygen tension than with arterial carbon dioxide tension or tidal volume.[3] Vidyasagar *et al* showed a threefold increase in pulmonary blood flow within three hours of surfactant replacement in 15 immature baboons with severe respiratory distress syndrome.[6] Pulmonary compliance also increased and the authors attributed the improvement in oxygenation to this, and the change in pulmonary blood flow to the presence of a left to right shunt across a patent ductus arteriosus.[6]

We speculated that increase in pulmonary blood flow might be responsible for the improved oxygenation as it amounted to about a 300% increase over baseline values. We estimated that a study of 20 infants would be large enough to detect a similar threefold increase in pulmonary blood flow. That we were unable to show such an increase may be explained in one of four ways. Firstly, baboons with respiratory distress syndrome do not behave like babies with respiratory distress syndrome, and secondly that the methods of measuring pulmonary blood flow were different; Vidyasagar *et al* used a microsphere technique[6] and we used a pulsed Doppler ultrasound method. Thirdly, the surfactant preparations are not identical and clinical studies with our porcine surfactant did not show an increased incidence of patent ductus arteriosus.[7] An alternative explanation is that we entered too few babies into our study to show a change in pulmonary blood flow. If the change had been threefold, however (that is, of the same order as the change in oxygenation), then our study should have been able to detect this. Our results are consistent with a modest change in pulmonary blood flow, certainly much less than the change in oxygenation.

We believe that the acute change in oxygenation is caused by improvement in the ventilation:perfusion ratio and not to an immediate increase in pulmonary blood flow. Our findings suggest that improved compliance is a more likely explanation and confirm those of Jobe *et al* who measured cardiac output and pulmonary blood flow before and after treatment of premature lambs with surfactant and found no difference.[4] We did not make direct measurements of pulmonary compliance, and have assumed that it changed with reduced ventilator settings and alterations in the cardiorespiratory trace of transthoracic impedance. Daily *et al* showed an inverse correlation between compliance and

Table *Difference between control and treated babies in arterial:alveolar oxygen tension ratio, mean airway pressure ×ventilation rate, and pulmonary blood flow before and after instillation of surfactant*

	Treated babies (n=12)	*Control babies (n=8)*	*Difference*	*95% Confidence intervals*
Arterial:alveolar oxygen tension ratio:				
Before	0·12	0·12	0	−0·03 to +0·03
After 15 minutes	0·31	0·14	+0·17	+0·05 to +0·29
After one hour	0·31	0·14	+0·17	+0·01 to +0·33
After four hours	0·32	0·17	+0·15	−0·01 to +0·31
Product of mean airway pressure×ventilation rate:				
Before	447	440	+7	−198 to +212
After 15 minutes	374	490	−116	− 92 to +324
After one hour	355	479	−124	− 75 to +323
After four hours	285	478	−193	+ 10 to +376
Pulmonary blood flow (ml/kg/minute):				
Before	317	323	−6	−106 to +118
After 15 minutes	338	307	+31	− 99 to +161
After one hour	309	279	+30	− 66 to +126
After four hours	327	268	+59	− 25 to +143

transthoracic impedance.[11] Further studies are needed of direct measurement of lung compliance in babies having surfactant replacement. Simultaneous measurements of lung compliance and pulmonary blood flow would help to clarify the mechanisms of the acute effect of exogenous surfactant and may also help to predict the long term response of individual babies.

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References

[1] Jobe A, Ikegami M, Glatz T, Yoshida Y, Diakomanolis E, Padbury J. Duration and characteristics of treatment of premature lambs with natural surfactant. *J Clin Invest* 1981;**67**:370–5.

[2] Jobe A, Ikegami M, Jacobs H, Jones S. Surfactant and pulmonary blood flow distributions following treatment of premature lambs with natural surfactant. *J Clin Invest* 1984;**73**:848–56.

[3] Jacobs H, Jobe A, Ikegami M, Glatz T, Jones SJ, Barajas L. Premature lambs rescued from respiratory failure with natural surfactant: clinical and biophysical correlates. *Pediatr Res* 1982;**16**:424–9.

[4] Jobe A, Jacobs H, Ikegami M, Jones S. Cardiovascular effects of surfactant suspensions given by tracheal instillation to premature lambs. *Pediatr Res* 1983;**17**:444–8.

[5] Clyman RI, Jobe A, Heymann M, *et al.* Increased shunt through the patent ductus arteriosus after surfactant replacement therapy. *J Pediatr* 1982;**100**:101–7.

[6] Vidyasagar D, Maeta H, Raju TN, *et al.* Bovine surfactant (surfactant TA) therapy in immature baboons with hyaline membrane disease. *Pediatrics* 1985;**75**:1132–42.

[7] McCord FB, Curstedt T, Halliday HL, McClure G, Reid MMcC, Robertson B. Surfactant treatment and incidence of intraventricular haemorrhage in severe respiratory distress syndrome. *Arch Dis Child* 1988;**63**:10–6.

[8] Noack G, Berggren P, Curstedt T, *et al.* Severe neonatal respiratory distress syndrome treated with the isolated phospholipid fraction of natural surfactant. *Acta Paediatr Scand* 1987;**76**:697–705.

[9] Alverson DC, Eldridge M, Dillon T, Yabek SM, Berman W Jr. Non-invasive pulsed Doppler determination of cardiac output in neonates and children. *J Pediatr* 1982;**101**:46–50.

[10] Bohn DJ, James I, Filler RM, *et al.* The relationship between $PaCO_2$ and ventilation parameters in predicting survival in congenital diaphragmatic hernia. *J Pediatr Surg* 1984;**19**:666–71.

[11] Daily W, Olsson T, Victorin L. Transthoracic impedance: V. Effects of early and late clamping of the umbilical cord with special reference to the ratio air-to-blood during respiration. *Acta Paediatr Scand* 1970;**207**(suppl):57–72.

[12] Gardner MJ, Altman DG. Confidence intervals rather than p values: estimation rather than the hypothesis testing. *Br Med J* 1986;**292**:746–50.

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Auditory brain stem responses in infants with posthaemorrhagic ventricular dilatation

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SUMMARY Nineteen infants with posthaemorrhagic ventricular dilatation had auditory brain stem responses measured during the period of maximal ventricular dilatation. These showed various patterns ranging from normal, through various abnormalities, to complete absence of responses. When serial auditory brain stem responses were studied in parallel with the evolution of posthaemorrhagic ventricular dilatation it was seen that the abnormalities of auditory brain stem response usually resolved irrespective of the persistence or progression of ventricular dilatation. No correlation was found between cerebrospinal fluid pressure and prolonged interpeak intervals on the auditory brain stem response. In three patients with posthaemorrhagic ventricular dilatation improvement in the auditory brain stem response occurred when cerebrospinal fluid was withdrawn. Intermittent withdrawal of cerebrospinal fluid (by ventricular tap or lumbar puncture) in two of these infants was followed by improvement in the auditory brain stem response after a period of 24 hours (but not sooner). In one infant born at full term improvement in the auditory brain stem response was noted one week after shunting.

Disturbances in auditory brainstem responses have been reported in disorders associated with ventricular dilatation in both infants and children,[1-3] and the use of the auditory brain stem response to assess the possible impact of ventricular dilatation on the brainstem has been described.[1-4]

The aim of the present study was to evaluate the auditory brain stem response abnormalities in infants with posthaemorrhagic ventricular dilatation and to determine whether such abnormalities could be altered by drainage of cerebrospinal fluid.

Patients and methods

Of the 855 infants admitted to the Hammersmith Hospital neonatal intensive care unit between October 1981 and April 1984, 145 had periventricular haemorrhage diagnosed on ultrasound scanning during the first week of life; 18 of these (12%) developed posthaemorrhagic ventricular dilatation. Two further infants who were transferred to the unit with established posthaemorrhagic ventricular dilatation at the ages of 2 and 8 weeks, respectively were included in the study. Of these 20 infants, 19 were fit for testing. The remaining infant was critically ill and considered unsuitable for assessment of auditory brain stem response; he subsequently died.

The diagnosis of posthaemorrhagic ventricular dilatation was made when two or more measurements of ventricular width on ultrasonography exceeded by at least 4 mm the 97th centile of the measurements appropriate for the gestational age determined by Levene.[5]

Of the 19 infants with posthaemorrhagic ventricular dilatation, 16 were preterm (gestational ages 26–34 weeks) and three had been born at full term (38–39 weeks). Gestational ages were calculated from gestational assessment[6] and the dates of the mother's last menstrual period, which agreed within one week in all cases.

The recordings of auditory brain stem response were usually made while the infant was lying prone with one ear on the mattress. An earphone was held gently over the other ear taking care not to occlude or collapse the external auditory canal, which may result in appearances simulating a conductive lesion. Rarefaction clicks, 100 μs square waves, at a rate of 10/s were made into one ear through a TDH-39 earphone. Each response was calculated from a total of 1024 to 2048 stimuli. Two machines were used; the first a Medelec MS6 model using a bandpass filter of 250–1600 Hz, the second a Medelec Sensor machine using a filter of 300–3000 Hz. Three standard electrodes were used, the active electrode was placed over the vertex (M1), and the reference electrode over the mastoid process ipsilateral to the stimulated ear. The contralateral mastoid process was used for the ground electrode (M2). A fourth electrode was sometimes applied to the forehead and used for grounding while M2 was recording the contralateral ear in a second channel. Due to limitation of time and availability of the babies, and to avoid excessive handling, we found it more convenient to use three electrodes most of the time. Contact impedances were generally of the order of 1000 to 2000 ohms, but on rare occasions were as high as 6000 ohms.

Intensities of 80 and 60 dB were routinely used. These intensities were altered as needed for particular subjects and circumstances, and according to the responses obtained. If the responses were poor, intensities were increased by increments of 10 dB. If the responses were good with well identified wave components the stimulus was decreased to 40 dB. All recordings were repeated to check for consistency.

Normal control data for each machine and setting were derived from two previous studies carried out in this unit.[7 8]

The cerebrospinal fluid pressure in nine of the 19 infants was also studied.[9] In six infants this was done within two of hours of their auditory brain stem responses having been measured. The pressures were recorded by direct measurement during lumbar or ventricular taps with a Gaeltic pressure transducer attached to the needle in the ventricular of subarachnoid space. The upper limit of normal cerebrospinal fluid pressure was taken as 0·8 kPa (6 mm Hg).

The four aspects studied were: the evolution of the abnormalities in auditory brain stem response in relation to the occurrence of periventricular haemorrhage and intraventricular haemorrhage and to the development and resolution of the ventricular dilatation (n=12); the assessment of the auditory brain stem responses at the time of maximum ventricular dilatation (n=19); the general association between abnormalities in auditory brain stem response and cerebrospinal fluid pressure (n=9); and the changes in auditory brain stem response in response to withdrawal of cerebrospinal fluid by either shunting, or periodic drainage by lumbar puncture or ventricular tapping (n=7).

Results

EFFECTS OF THE EVOLUTION OF POSTHAEMORRHAGIC VENTRICULAR DILATATION ON THE AUDITORY BRAIN STEM RESPONSE

Eleven preterm infants and one born at full term were studied. In the preterm infants periventricular haemorrhage or intraventricular haemorrhage of variable severity were diagnosed during the first week of life. During the same time auditory brain stem responses were recorded and were abnormal in all 11 infants. The haemorrhages and sequential auditory brain stem responses were measured and showed improvement at a time when the ventricular dilatation was still present on the scans, or even increasing in size. In five of the 11 preterm infants the auditory brain stem responses returned to normal although there was still pronounced dilatation of the ventricles. Fig 1 gives an example of these five infants. In three the auditory brain stem responses remained abnormal at the ages of 10, 14, and 20 weeks, respectively. Though the ventricles decreased in size during this period, they still remained moderately dilated.

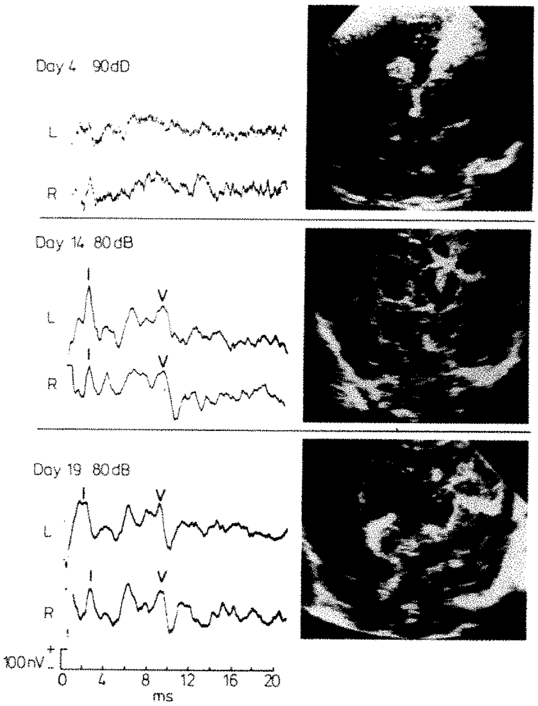


Fig 1 *An infant born at 32 weeks' gestation developed bilateral intraventricular and periventricular haemorrhages on day 3. Auditory brain stem responses on day 4 were abnormal (latencies, intervals, and waveforms) at 90 dB on each side. By the second week the auditory brain stem response showed pronounced improvement on wave forms but the I-V interval remained prolonged. Ventricular dilatation and cyst formation were now present on ultrasonography. By day 19, the auditory brain stem response had reverted to normal, but there had been further dilatation on ultra sonography. Click stimuli used in all traces at 10/s (rarefaction phase).*

In one infant the auditory brain stem response remained abnormal and the child was referred to hearing specialists. Two infants died at the ages of 2 and 4 weeks, respectively.

A single infant born at full term with posthaemorrhagic ventricular dilatation was also studied and showed an initially normal auditory brain stem response. Ventricular dilatation increased progressively over the next 10 weeks. A repeat recording early in this period remained normal. Subsequent auditory brain stem responses measured at the age of 3 months showed deterioration.

EFFECTS OF POSTHAEMORRHAGIC VENTRICULAR DILATATION ON THE AUDITORY BRAIN STEM RESPONSE AT TIME OF MAXIMUM VENTRICULAR DILATATION

Auditory brain stem responses were measured in 19 infants during the phase of maximum dilatation of the ventricles. Five records were normal and 14 were abnormal. The abnormal tracings showed various patterns. Two infants had 'severe' impairment: absent responses in one, and appreciably reduced wave amplitude in the other (figs 2a and b, respectively). Two infants had 'peripheral' abnormalities including absent wave amplitude or wave 1 more than two standard deviations from the mean (fig 2c). Five infants had 'central' abnormalities. Three showed prolonged I–V interpeak intervals (more than two standard deviations from the mean), one with an absent wave V, and one with an abnormal V:I amplitude ratio (figs 2d and e). Two infants had short I-V interpeak intervals but normal wave configuration. Three infants had abnormal and non-reproducible traces on repeated testing. These were classified separately.

EFFECTS OF CEREBROSPINAL FLUID PRESSURE ON THE AUDITORY BRAIN STEM RESPONSE

Nine infants had serial cerebrospinal fluid pressures and auditory brain stem responses recorded, six of whom had the auditory brain stem responses recorded on the same day and within a few hours of recording the cerebrospinal fluid pressures. In these six infants therefore (five preterm and one born at full term) a total of 21 direct cerebrospinal fluid pressure measurements were correlated with the auditory brain stem response measurements that were recorded simultaneously. There was no correlation between the cerebrospinal fluid pressure and the presence of auditory brain stem response abnormalities as assessed by the I-V interpeak interval (fig 3).

EFFECTS OF CEREBROSPINAL FLUID DRAINAGE ON THE AUDITORY BRAIN STEM RESPONSE

In seven infants the auditory brain stem responses were studied before and after drainage of cerebrospinal fluid. In all seven the auditory brain stem responses were abnormal before the drainage was carried out. Repeat measurement of auditory brain stem responses after two to six hours showed little change, but auditory brain stem responses measured 24 hours after drainage showed an increase in amplitude in two of the four cases where this was tested (fig 4).

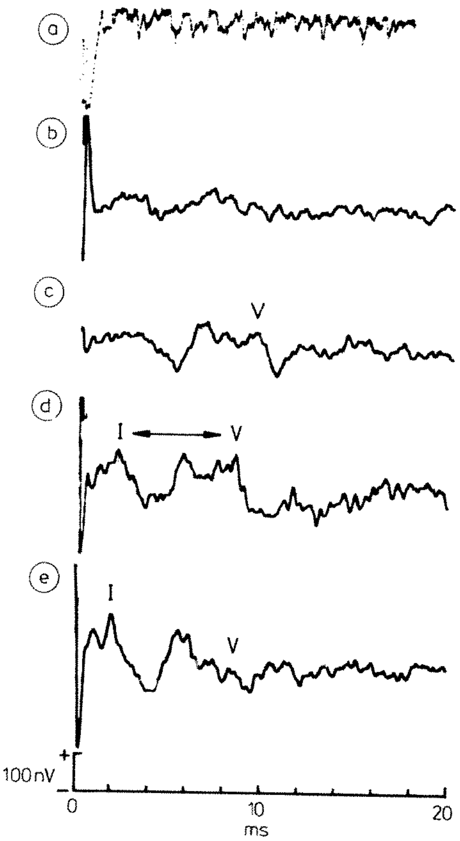


Fig 2 *Auditory brain stem response recordings showing various types of abnormalities. (a) Absent responses at 80 dB. (b) Severely reduced response at intensity: 80 dB. (c) Absent wave 1. (d) Prolonged interpeak intervals between waves I-V in an infant, postmenstrual age 34 weeks; interpeak interval is 6·2 ms—that is, more than two SD from mean. (e) Poor wave V with abnormal V:1 ratio. Click stimuli used in all traces at a rate 10/s and the intensities used in traces c–e were 60 dB.*

Four infants had shunts inserted. Auditory brain stem responses measured before the shunts were inserted were normal in one, unequivocally abnormal in another, and showed a pattern of uncertain importance in the remaining two, with normal wave

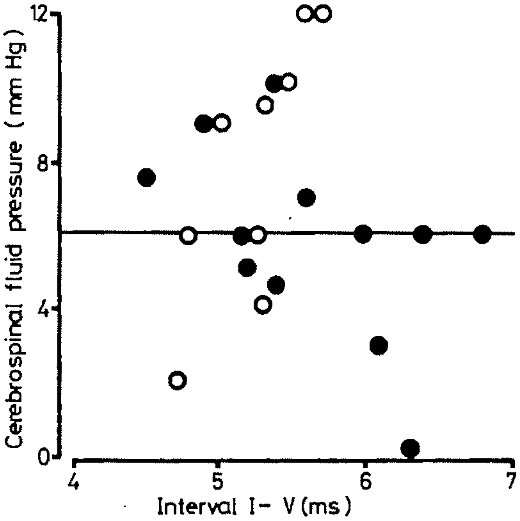


Fig 3 *Simultaneous cerebrospinal fluid pressures and interpeak intervals I-V recorded on 12 occasions from five preterm infants (black circles), and on nine occasions from one infant born at full term (white circles). The upper limit of normal cerebrospinal fluid pressure is shown by the horizontal line.*

morphology and hearing thresholds, but interpeak intervals shorter than were appropriate for their ages. The shunting procedure had no effect on the auditory brain stem response in one patient in whom the auditory brain stem response was normal before shunting or in two patients who had abnormalities in the auditory brain stem response of uncertain importance. In the one infant born at full term, however, the abnormal auditory brain stem response recorded before the shunt reverted to normal within a week of the shunt being inserted (fig 5).

Discussion

Previously reported abnormalities in auditory brain stem responses in children with hydrocephalus were found in much older subjects[3] [10] or in children of unspecified age.[1] In some instances the cause of the hydrocephalus was not documented. Previous investigators[1] [2] have showed that in the presence of ventricular dilatation there were increased I-V interpeak intervals that they attributed to increased intracranial pressure. We know of no previous publications on the association between the changes in auditory brain stem response and the development of posthaemorrhagic ventricular dilatation, the

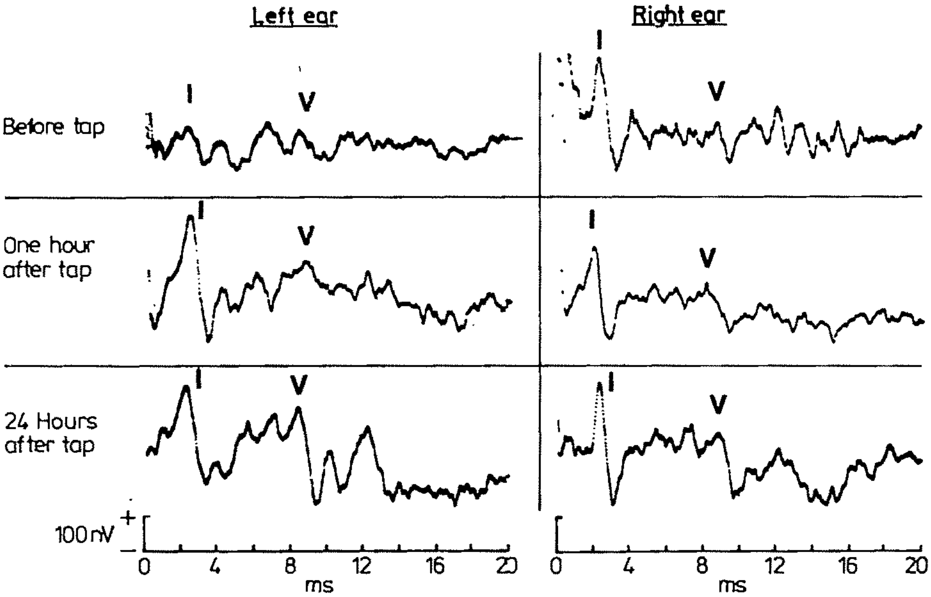
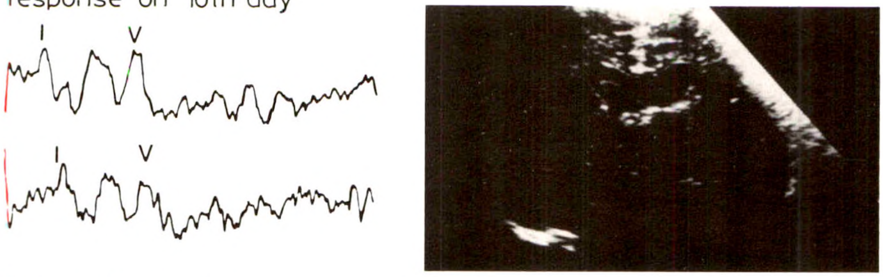
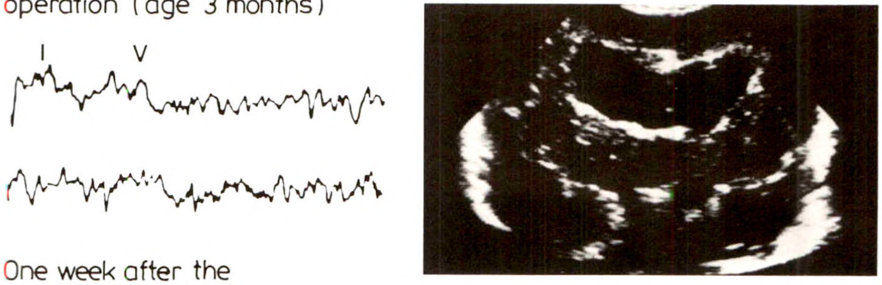


Fig 4 *Effect of ventricular tap on the auditory brain stem response of a baby girl born at 29 weeks' gestation with right sided periventricular and intraventricular haemorrhages on ultrasonography. Before drainage the auditory brain stem responses (both left and right) showed abnormal waveforms and latencies. One hour after cerebrospinal fluid tap there was little change in the auditory brain stem response (a unilateral increase in the amplitude of wave 1 on the left). The responses remained abnormal. When auditory brain stem responses were measured again 24 hours later, there was considerable improvement, particularly on the left, where both latencies and I-V interval had returned to normal limits. The right side remained abnormal (prolonged I-V interval and abnormal waveforms). All stimuli at 80 dB and 10 clicks/s.*

(a) Initial auditory brain stem response on 10th day



(b) Before the shunting operation (age 3 months)



(c) One week after the shunting operation

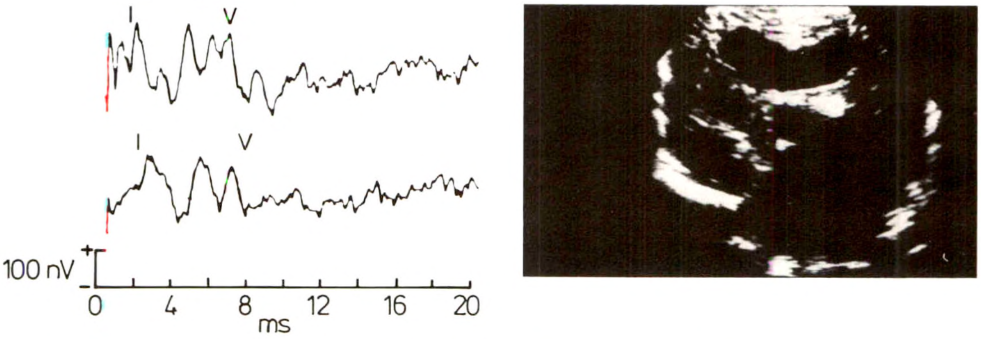


Fig 5 *Effect of shunting on the auditory brain stem response in an infant born at full term with posthaemorrhagic ventricular dilatation. (a) Initial auditory brain stem responses show normal latency and wave configuration at 60 and 40 dB. Ultrasonography shows some ventricular dilatation. (b) Effects of progressive ventricular dilatation lasting over three months (pronounced deterioration of wave configuration, and disappearance of responses at 40 dB). Both lateral ventricles show pronounced dilatation on ultrasonography carried out on the same date as the auditory brain stem response. (c) Auditory brain stem response measured one week after shunting showing reversion to normal. The ultrasound scan showed that the ventricles, while still dilated, had diminished in size. All stimuli at 10 clicks/s.*

effects of intermittent drainage of cerebrospinal fluid, or the rise in intracranial pressure. In the present study, we found no correlation, either positive or negative, between the I-V interpeak interval and the intracranial pressure in preterm infants, and this is probably due to better adaptation of the immature brain to increased intracranial pressure.

The mechanisms whereby the brains of preterm infants can adapt to increased intracranial pressure are open to speculation. One may be the balance between increased ventricular volume and the resorption of extracerebral cerebrospinal fluid. When the limits of compensation are reached a sustained rise in the intracranial pressure will ensue giving rise to the classic signs of increased intracranial pressure with increasing head circumference, tense anterior fontanelle, and suture diastasis.[11]

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Necropsy studies have also shown that in preterm infants with posthaemorrhagic ventricular dilatation considerable degrees of dilatation can occur before there is any rise in intracranial pressure.[12 13] Periventricular oedema, produced by cerebrospinal fluid extravasating through ruptures in the ependyma, may compress periventricular capillaries and cause ischaemic injury.[14] Hill and Volpe have also stressed that the immature state of the periventricular tissue and the fact that it has been compromised by a variety of hypoxic and ischaemic insults may influence its adaptation to increased intracranial pressure.[15]

Another factor influencing this adaptation is the comparatively large subarachnoid space within the cranium of the premature infant.[12 16] Among preterm infants there is a wide variation in the size of the brain and of the extracerebral space depending on the pressure to which the head has been subjected in the uterus and on variations in fetal maturity and nutrition.[13] These facts are probably relevant to our findings concerning the association between intracranial pressure and I-V interpeak intervals.

The data should probably not be extrapolated to infants born at full term or to older children. Our data for our single infant born at full term were similar to those previously reported. Nagao *et al* reported associated reductions in the amplitudes of waves IV and V, and prolonged latencies of waves III, IV, and V after experimentally induced intracranial hypertension in cats (the intracranial pressure being raised by expansion of a supratentorial balloon).[17] Although increased intracranial pressure and abnormalities in auditory brain stem response have been described in animals and in humans,[7–19] Kraus *et al*[3] found ABR abnormalities in auditory brain stem response in patients after shunts had been inserted in the absence of increased intracranial pressure.

Much less is known about the specific associations between intracranial pressure and brain stem function. Goodman and Becker studied the vascular abnormalities in the brain stem induced by intracranial hypertension in cats.[20] They found that as intracranial pressure rose there was a parallel progression of vascular disturbances, first in the microcirculation and finally in the macrocirculation. Such reversible ischaemic changes in the brain stem caused by alterations in blood flow may account for the changes in auditory brain stem response on intermittent withdrawal of cerebrospinal fluid seen in some of our patients. In an experiment on cats, Nagao *et al* found progressive reduction in the amplitude of waves IV and V as the intracranial pressure was gradually increased by balloon inflation, followed by progressive recovery during a three hour period after the balloon had been deflated.[21] They suggested that these changes were due to direct mechanical pressure. The changes were clearly not irreversible. Recently Little *et al* showed that intraoperative auditory brain stem response recording during surgical treatment of a basilar artery aneurysm resulted in contralateral amplitude reduction of waves III-V only during pontine retraction.[22] The effect was transient and the patient completely recovered. Another explanation for our data could be the recovery of brain cells from interstitial oedema (or effused cerebrospinal fluid), a recovery reflected in an increase in wave amplitude.

This study has shown that in premature infants abnormalities in auditory brain stem responses may resolve irrespective of the persistence or progression of ventricular dilatation. No correlation was found between cerebrospinal fluid pressure and certain abnormalities of auditory brain stem responses (such as prolonged interpeak interval) but an improvement of the auditory brain stem response—especially of the amplitude—could occur after drainage of cerebrospinal fluid.

A larger number of infants born at full term needs to be studied to observe whether their response to ventricular dilatation and raised intracranial pressure is indeed different from that in preterm infants.

References

[1] Starr A, Amlie RN. The evaluation of the newborn brainstem and cochlear functions by auditory brainstem potentials. In: Krobkin R, Guilleminault C, eds. *Progress in perinatal neurology*. Baltimore: Williams and Wilkins, 1981:65–83.

[2] De Vlieger M, Sadikoglu S, Van Eijndhoven JHM, Atac M. Visual evoked potentials, auditory evoked potentials and EEG in shunted hydrocephalic children. *Neuropediatrics* 1981;**12:** 55–61.

[3] Kraus N, Ozdamar O, Stein L, Reed N. Absent auditory brainstem response: peripheral hearing loss or brainstem dysfunction. *Laryngoscope* 1984;**94:**400–6.

[4] Stockard JE, Stockard JJ. Brainstem auditory evoked potentials in normal and otoneurologically impaired newborns and infants. In: Henry CE, ed. *Current clinical neurophysiology—update on EEG and evoked potentials.* Amsterdam: Elsevier, 1981:9–71.

[5] Levene MI. Measurement of the growth of the lateral ventricles in preterm infants with real-time ultrasound. *Arch Dis Child* 1981;**56:**416–24.

[6] Dubowitz LMS, Dubowitz V, Goldberg C. Clinical assessment of gestational age in the newborn infant. *J Pediatr* 1970;**77:**1–10.

[7] Fawer C, Dubowitz LMS. Auditory brainstem responses in neurologically abnormal infants. *Neuropediatrics* 1982;**14:**88–92.

[8] Lary S. Auditory brainstem responses in normal and abnormal preterm and full-term infants. London: University of London 1986. 310 pp. (Thesis.)

[9] Kaiser AM, Whitelaw AGL. Cerebrospinal fluid pressure during post-haemorrhagic ventricular dilatation in newborn infants. *Arch Dis Child* 1985;**60:**920–4.

[10] Stein L, Ozdamar O, Kraus N, *et al.* Follow-up infants screened by auditory brainstem response in the neonatal intensive care unit. *J Pediatr* 1983;**103:**447–53.

[11] Volpe JJ. Neonatal intracranial haemorrhage: pathophysiology, neurophysiology and clinical features. *Clin Perinatol* 1977;**4:** 77–101.

[12] Larroche JC. *Developmental pathology of the neonate.* Amsterdam: Excerpta Medica, 1977:368–98.

[13] Wigglesworth J. *Perinatal pathology. Major problems in pathology 15.* Philadelphia: WB Saunders, 1984.

[14] Wozniak M, McLone DG, Riamondi AJ. Micro and macrovascular changes as the direct cause of parenchymal destruction in congenital murine hydrocephalus. *J Neurosurg*1975;**43:**535–45.

[15] Hill A, Volpe JJ. Seizures, hypoxic-ischaemic brain injury and intraventricular haemorrhage in the newborn. *Ann Neurol* 1981;**10:**109–21.

[16] Pape KE, Wigglesworth J. *Haemorrhage, ischaemia and the perinatal brain. Clinics in developmental medicine 69/70.* London: William Heinemann, 1979.

[17] Nagao S, Roccaforte P, Moody RA. Acute intracranial hypertension and auditory brainstem responses. Part 1: changes in the auditory brainstem and somatosensory evoked responses in intracranial hypertension. *J Neurosurg* 1979;**51:**669–76.

[18] Nagao S, Sunami N, Tsutsui T, *et al.* Serial observations of brainstem function in acute intracranial hypertension by auditory brainstem responses—a clinical study. In: Ishii S, Nagai H, Brook M, eds. *Intracranial pressure V.* Berlin: Springer Verlag, 1983:474–9.

[19] Benna P, Gilli M, Ferrero P, Bergamasco B. Brainstem auditory evoked potentials in supratentorial tumours. *Electroencephalogr Clin Neurophysiol* 1982;**54:**8–9

[20] Goodman SJ, Becker DP. Vascular pathology of the brain stem due to experimentally increased intracranial pressure: changes noted in the micro and macrocirculation. *J Neurosurg* 1973;**39:**601–9.

[21] Nagao S, Roccaforte P, Moody RA. Acute intracranial hypertension and auditory brainstem responses. Part 2: The effects of brain-stem movement on the auditory brain-stem responses due to transtentorilal herniation. *J Neurosurg* 1980;**51:**846–51.

[22] Little JR, Larkins MV, Luders H, Hahn JF, Erenberg G. Fusiform basilar artery aneurysm in a 33 month old child. *Neurosurgery* 1986;**19:**631–4.

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Management of posthaemorrhagic hydrocephalus with a subcutaneous ventricular catheter reservoir in premature infants

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SUMMARY A subcutaneous ventricular catheter reservoir was inserted between the 16th and 28th (median 23rd) day of life in 13 premature infants (median birth weight 1220 g, range 780–2110) for the treatment of posthaemorrhagic hydrocephalus. Aspiration of cerebrospinal fluid (median 6 ml, range 1–15) one to four (median two) times a day for 6 to 90 (median 40) days controlled the hydrocephalus as judged by clinical and ultrasonographic criteria. There were no serious complications, except skin breakdown in one patient. Intracranial pressure measured in five patients through the reservoir showed the efficacy of the treatment, as well as the unreliability of clinical and ultrasonographic criteria in determining the amount of cerebrospinal fluid that should be aspirated daily.

Management of posthaemorrhagic hydrocephalus in premature infants during the first weeks of life is difficult because of the low body weight and the associated serious medical problems in these severely compromised patients. Surgical methods of controlling the intracranial pressure include serial lumbar punctures,[1-3] external ventricular drainage (usually through a subcutaneously tunnelled catheter)[2 4 5] insertion of a ventriculoperitoneal shunt,[6] and placement of a subcutaneous ventricular catheter reservoir.[7 8]

Since 1982 we have used a ventricular catheter with subcutaneous reservoir for the treatment of posthaemorrhagic hydrocephalus. In five of our patients the intracranial pressure was measured through the reservoir. The aim of this retrospective study was to assess the efficacy and the complications of this particular treatment, and of measuring intracranial pressure through the reservoir.

Patients and methods

Between 1982 and 1987 a ventricular catheter with a subcutaneous reservoir (Holter-Hausner International) was placed in each of 13 premature infants with posthaemorrhagic hydrocephalus (table 1). The insertion of the reservoir was considered necessary if at least one of the criteria given in table 2 was met. The procedure took about 30 minutes under general anaesthesia and was done in the operating theatre. The ventricular catheter was positioned in the frontal horn of the right lateral ventricle, and the

Table 1 *Characteristics of premature infants and courses of treatment of posthaemorrhagic hydrocephalus with subcutaneous reservoir (n=13)*

	Median	*(range)*
Gestational age (weeks)	29	(26–33)
Birth weight (g)	1070	(740–2130)
Haemorrhage grade[10]	3	(2–4)
Age at insertion of reservoir (days)	23	(16–28)
Weight at insertion of reservoir (g)	1220	(780–2110)
Period during which aspiration of cerebrospinal fluid carried out (days)	40	(6–90)
No of aspirations:		
No/day for each patient	2	(1–4)
Total No/each patient	59	(12–309)
Volume of cerebrospinal fluid aspirated (ml):		
Volume at each aspiration	6	(1–15)
Total volume/each patient	477	(76–1917)

Table 2 *Criteria for insertion of a subcutaneous reservoir*

- Increase in head circumference of more than 1 cm/week
- Progressive ventricular dilatation on ultrasound scans
- Obstructive posthaemorrhagic hydrocephalus (failure to control posthaemorrhagic hydrocephalus by lumbar punctures)
- Severe deterioration during lumbar puncture (for example, bradycardia, or apnoea)

dome of the reservoir was positioned over the parietal bone. Prophylactic antibiotics were not given.

The reservoir was tapped with a small gauge (25 or 27) scalp vein needle, the operator wearing sterile gloves, and having carefully disinfected the skin. The aspirated volume of cerebrospinal fluid and the frequency of tapping were considered to be adequate if the following criteria were fulfilled: (i) increase in head circumference of less than 1 cm/week, (ii) unchanged or decreasing size of ventricles on ultrasound scans, (iii) normal tension of the fontanelle, and normal position of the cranial sutures. Occipitofrontal head circumference was measured and the fontanelle and the cranial sutures were palpated daily by the same person. Ultrasound scans of the brain with estimation of the ventricular size, as well as cell count, protein and glucose concentrations, and bacterial cultures of specimens of cerebrospinal fluid were carried out at least once a week. White blood cell count, and serum electrolyte and total serum protein concentrations were measured every other week.

In five patients a pressure transducer (Sensonor) was used to measure the intracranial pressure through the reservoir before and after aspiration of cerebrospinal fluid. An intracranial pressure of less than 6 mm Hg was considered to be normal.[9]

For evaluation of the risk of the treatment the following possible complications were recorded: infection of the reservoir or sepsis (the presence of pathogens on culture of the cerebrospinal fluid), breakdown of the skin overlying the reservoir, intraventricular haemorrhage after aspiration of cerebrospinal fluid, leak from the reservoir, obstruction of the ventricular catheter, or low serum sodium (<135 mmol/l) or total protein (<40 g/l) concentrations.

Results

The essential data concerning the treatment are shown in table 1. Six patients weighed 1000 g or less at the time of insertion of the reservoir. Daily aspirations of the reservoir for more than 40 days were carried out in seven patients.

In all 13 patients, normal increase of occipitofrontal head circumference, unchanged or decreasing ventricular size, normal tension of the fontanelle, and normal position of cranial sutures were achieved. In 12 patients tapping was continued until a ventriculoatrial or ventriculoperitoneal shunt could be inserted with a low risk of complications. At the time of placement of the shunt, the median weight was 2320 g (range 1500–4500) and protein concentration in the cerebrospinal fluid was less than 1·5 g/l. In one patient the posthaemorrhagic hydrocephalus resolved spontaneously and no further treatment was necessary. None of the patients died.

Intracranial pressure was measured through the reservoir before and after aspiration of cerebrospinal fluid in five patients on 47 occasions (table 3). Aspiration of cerebrospinal fluid caused a decrease in intracranial pressure of between 2 and 11 (median 3) mm Hg. In fourteen of the 47 measurements before, and in two of the 47 measurements after aspiration of cerebrospinal fluid, the intracranial pressure was raised (that is, above 6 mm Hg). In three patients intracranial pressure was raised and returned to normal or was unchanged within the normal range; in another patient, the raised intracranial pressure did not decrease during the treatment, and subsequently a ventriculoatrial shunt was inserted. The clinical course and the ventricular size on ultrasound scans corresponded with the degree of intracranial pressure in these four patients. In one patient, however, intracranial pressure was raised to 14 mm Hg, although clinical signs and ventricular size on ultrasound scans did not show any signs of increasing intracranial pressure. This patient also had a ventriculoatrial shunt inserted.

The following complications occurred: skin breakdown (n=1)—the reservoir was exchanged and prophylactic antibiotics were started; hyponatraemia (n=8), and hypoproteinaemia (n=2). In one patient erythrocytes were found in the cerebrospinal fluid during the fourth and fifth week after the reservoir had been inserted, but new intraparenchymal or intraventricular haemorrhages were not detectable on ultrasound examination. Neither a leak from the reservoir nor obstruction of the ventricular catheter was seen, nor did any infection occur that could be associated with the reservoir.

Discussion

The results of this study show that posthaemorrhagic hydrocephalus can be successfully treated with a subcutaneous ventricular catheter reservoir. Our

Table 3 *Intracranial pressure measured through a subcutaneous reservoir on 47 occasions in five premature infants with posthaemorrhagic hydrocephalus before and after aspiration of cerebrospinal fluid*

	Median	*(range)*
Age (days)	26	(19–89)
Intracranial pressure (mm Hg):		
Before aspiration	4	(1–19)
After aspiration	1	(0–8)
Volume of cerebrospinal fluid aspirated (ml)	5	(2–7)

findings confirm those of previous studies[7 8] that also showed decreasing ventricle size on computed tomograms of the brain after aspiration of cerebrospinal fluid through a subcutaneous reservoir.

A common problem in the treatment of posthaemorrhagic hydrocephalus with a subcutaneous reservoir is to know how often and how much to tap. In the present study the frequency and volume were determined from clinical signs and the size of the ventricles on ultrasound scans. Ultrasound estimation of ventricle size is, however, sometimes difficult in infants with posthaemorrhagic hydrocephalus, especially if porencephalic cysts or asymmetrical ventricular enlargement have developed. Moreover tension of the fontanelle and increasing head circumference are not always sensitive signs of raised intracranial pressure.[11 12]

We therefore repeatedly measured the intracranial pressure through the reservoir in five infants (table 3). Most measurements of intracranial pressure were within the normal range, and this shows the efficacy of the treatment that was guided by clinical and ultrasonographic criteria. Some of the high measurements of intracranial pressure were found at the beginning of the treatment and decreased subsequently. In general, clinical and ultrasonographic signs corresponded with the degree of the intracranial pressure. We did, however, find high intracranial pressure in one patient though clinical signs and ultrasound scans did not indicate any increase in intracranial pressure. The control and guidance of the treatment by only clinical and ultrasonographic criteria, therefore, is not sufficient in some patients, but may be improved by the measurement of intracranial pressure through the reservoir. Intermittent peaks of intracranial pressure between the taps cannot be ruled out even if intracranial pressure is normal at the time of aspiration of cerebrospinal fluid. Continuous monitoring of intracranial pressure could probably further increase the efficacy of the treatment until clinical and ultrasonographic criteria are stable.

We found skin breakdown in one patient four weeks after insertion of the reservoir. At this time the daily volume of cerebrospinal fluid being aspirated had been decreased in an attempt to wean the patient off the taps. Rising intracranial pressure with subsequent head growth had probably increased the vulnerability of the skin overlying the reservoir.

In one of our patients we found fresh erythrocytes in the cerebrospinal fluid during the fourth and fifth weeks after the insertion of the reservoir. On ultrasound scans neither new intraventricular nor intraparenchymal haemorrhages were detectable. Rupture of a small intracranial blood vessel cannot be ruled out, however. The intermittent aspiration of cerebrospinal fluid caused fluctuations in intracranial pressure of up to 11 mm Hg. Thus rupture of intracranial vessels may be the result of rapid aspiration of cerebrospinal fluid.

Hyponatraemia was found in eight infants, and hypoproteinaemia in two. The extent of hypoproteinaemia and hyponatraemia were not associated with the amount of cerebrospinal fluid that was being aspirated, but sodium and protein supplementation were not standardised in these infants. Sodium and protein depletion, which are theoretically possible if large amounts of cerebrospinal fluid are aspirated, can be avoided by giving sodium and protein supplements.

Obstruction of the ventricular catheter did not occur nor was it seen in another series of patients with posthaemorrhagic hydrocephalus treated with a ventricular catheter reservoir.[8]

Cerebrospinal fluid infections did not occur in spite of frequent tappings during a period of up to 90 days (table 1). Marlin[7] reported two infections associated with insertion of the reservoir in a group of 12 patients with a maximum period of treatment of 101 days. In another study[8] no infections of the reservoir were seen in 20 patients with a maximum period of treatment of 48 days. Thus the risk of infection associated with the reservoir is low. External drainage with a subcutaneously tunnelled distal catheter[4 5] was also associated with a low risk of infection, but the period of treatment did not exceed 42 days. Infections associated with shunts occurred in 25% of infants with ventriculoperitoneal shunts.[6 13 14] In infants weighing more than 2000 g early insertion of a ventriculoperitoneal shunt has a low risk of infection,[6] but a high protein content or debris in the cerebrospinal fluid may cause obstruction of the ventricular catheter.

In most premature infants with posthaemorrhagic hydrocephalus, temporary measures for control of raised intracranial pressure are necessary until the insertion of an internal shunt can be carried out under optimal conditions after resolution of the patient's medical problems. Our data, together with other reports on the treatment of posthaemorrhagic hydrocephalus in small preterm infants, suggest the following approach: after the diagnosis of posthaemorrhagic hydrocephalus, serial lumbar punctures should be performed. If lumbar punctures do not succeed in controlling the intracranial pressure or if the infant deteriorates critically during the puncture, a subcutaneous ventricular catheter reservoir should be inserted if the baby weighs less than 2000 g. The volume and frequency of tapping of the reservoir is determined by clinical signs (tense fontanelle and increasing head circumference) and ultrasound scans. Direct intracranial pressure measurements through the reservoir may increase the efficacy and the safety of the treatment. A ventriculoatrial or ventriculoperitoneal shunt should be inserted if resolution of the posthaemorrhagic hydrocephalus has not taken place by the time the infant weighs 2000 g, and if the protein concentration of the cerebrospinal fluid is low.

References

[1] Papile LA, Burstein J, Burstein R, Koffler H, Koops BL. Posthemorrhagic hydrocephalus in low-birth-weight infants: treatment by serial lumbar punctures. *J Pediatr* 1980;**97**:273–7.
[2] Allan WC, Volpe JJ. Periventricular-intraventricular hemorrhage. *Pediatr Clin North Am* 1986;**33**:47–63.
[3] Kreusser KL, Tarby TH, Kovnar EK, Taylor DA, Hill A, Volpe JJ. Serial lumbar punctures for at least temporary amelioration of neonatal posthemorrhagic hydrocephalus. *Pediatrics* 1985;**75**: 719–24.
[4] Harbaugh RE, Saunders RL, Edwards WH. External ventricular drainage for control of posthemorrhagic hydrocephalus in premature infants. *J Neurosurg* 1981;**55**:766–70.
[5] Amato M, Guggisberg Ch, Kaiser G. Treatment of progressive posthemorrhagic hydrocephalus with temporary external ventricular drainage. *Helv Paediatr Acta* 1986;**41**:317–24.
[6] James HE, Bejar R, Gluck L, *et al.* Ventriculoperitoneal shunts in high risk newborns weighing under 2000 grams: a clinical report. *Neurosurgery* 1984;**15**:198–202.
[7] Marlin AE. Protection of the cortical mantle in premature infants with posthemorrhagic hydrocephalus. *Neurosurgery* 1980;**7**:464–8.
[8] McComb JG, Ramos AD, Platzker ACG, Henderson DJ, Segall HD. Management of hydrocephalus secondary to intraventricular hemorrhage in the preterm infant with a subcutaneous ventricular catheter reservoir. *Neurosurgery* 1983;**13**:295–300.
[9] Hill A. Intracranial pressure measurements in the newborn. *Clin Perinatol* 1985;**12**:161–78.
[10] Papile LA, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights of less than 1,500 gm. *Pediatrics* 1978;**92**:629–34.
[11] Kaiser AM, Whitelaw AGL. Intracranial pressure estimation by palpation of the anterior fontanelle. *Arch Dis Child* 1987;**62**: 516–17.
[12] Korobkin R. The relationship between head circumference and the development of communicating hydrocephalus in infants following intraventricular hemorrhage. *Pediatrics* 1975;**56**:74–7.
[13] Hahn YS, McLone DG, Raimondi AJ, Frisbe CL. Surgical outcome of preterm newborns with severe periventricular-intraventricular hemorrhage and post-hemorrhagic hydrocephalus. *American Society for Pediatric Neurosurgery: concepts in pediatric neurosurgery.* Basle: Karger, 1983.
[14] Scarff TB, Anderson DE, Anderson CL, Caldwell CC. Complications of ventriculoperitoneal shunts in premature infants. *American Society for Pediatric Neurosurgery: concepts in paediatric neurosurgery.* Basle: Karger, 1983.

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Commentary

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The early management of hydrocephalus after intraventricular haemorrhage in low birthweight, premature infants of low gestational age continues to give problems. Those who care for these tiny infants are still seeking the ideal method of treatment that will control the hydrocephalus but neither produce infection of the cerebrospinal fluid or damage the central nervous system. By inserting a ventricular catheter attached to a reservoir situated beneath the scalp, the authors achieved satisfactory control in 13 infants with gestational ages ranging from 26 to 33 weeks, and with birth weights of between 740 and 2130 gm by repeatedly tapping the reservoir, sometimes more than once a day, for periods lasting up to 90 days. While the necessity to carry out the taps and the volume of fluid removed was largely determined by the clinical signs and the size of the ventricles measured by ultrasound scanning, note was also taken of intracranial pressure measurements made through the reservoir before and after removal of the cerebrospinal fluid. The authors contend that measurement of the intracranial pressure probably increased the efficacy of their treatment.

In this small series satisfactory control of the hydrocephalus was achieved, but not without complications. Wound breakdown occurred in one case, and in another fresh blood was found in the cerebrospinal fluid after a tap. No infections or blockages of the ventricular catheters occurred, but it has to be stressed that this was an extremely small series. The method allows easy repeated removal of cerebrospinal fluid from the lateral ventricles and is a convenient way of measuring intracranial pressure, but it has the disadvantage that it requires an operation to be performed on a small and often frail premature infant, and insertion of a ventricular catheter does cause some local cortical damage. What the method does not permit is continuous removal of cerebrospinal fluid, and therefore—as the authors point out—the intracranial pressure is not smoothly controlled. Whether or not this is important for long term development of the brain has yet to be determined. Other methods of intermittent removal of cerebrospinal fluid from the ventricular system probably achieve results comparable to those reported by the authors.

Though the method described has some attractive features, in particular its use of intracranial pressure measurements, it still falls far short of the ideal, and the search will no doubt continue for a method with minimal complications that permits the raised intracranial pressure in premature infants with post-haemorrhagic hydrocephalus to be controlled smoothly until such time as they can have an indwelling cerebrospinal fluid diversion system inserted.

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Providing warmth for preterm babies by a heated, water filled mattress

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SUMMARY Seventeen healthy preterm babies had extra warmth provided in their cots by thermocontrolled, heated, water filled mattresses. As controls 17 babies of the same weight were nursed in air heated incubators. Both groups were studied for three weeks. No differences were found in minimal oxygen consumption (measured by indirect calorimetry), rectal and mean skin temperatures, or in daily weight gain. The babies were kept just as warm on the heated, water filled mattresses as in air heated incubators but the mattresses had the advantage of giving the mothers easy access to their babies.

Air heated incubators and thermocontrolled radiant heaters are most often used to warm preterm babies in modern neonatal care units. Both techniques are complex, expensive, and require a continuous electrical supply. These techniques are, therefore, not suitable for use in most developing countries. After recommendations from UNICEF, and a commission of the World Health Organisation, a heated, water filled mattress was developed as an alternative.[1]

The present study was set up to compare the long term thermal effects of nursing healthy preterm infants in cots with the heated, water filled mattresses, with those of a control group of infants treated in single walled, air heated incubators.

Patients and methods

Each infant was cared for after birth during a stabilisation period in an air heated incubator. Thirty four premature or low birth weight, or both, infants were included in the investigation when they had reached a weight of 1400 to 1999 g. Infants who were free from infections, cardiopulmonary diseases, and gross malformations were studied as soon as they no longer needed supplementary oxygen. Informed consent from one or both parents was obtained.

The 34 babies were randomly allocated to either a study group who were nursed in cots on heated, water filled mattresses (n=17) or to a control group who were maintained in air heated incubators (n=17). The study period ran from the day of randomisation to the day when the baby moved to an ordinary cot. This occurred for babies on the mattresses when the mattress temperature was 36°C, and for the incubator group when the air temperature in the incubator was 30°C, and the babies had normal, stable body temperatures.

The gestational age of all but two infants was determined from the early (16 to 17 weeks) ultrasonographic estimate of the fetal biparietal skull diameter. In two infants ultrasonographic examinations had not been performed and therefore postnatal maturity evaluation by Finnström's method was used.[2]

There were no significant differences between the groups in the mean age of the mothers, parity, or the gestational age. In both groups there were large proportions of caesarean sections—seven in the mattress group and 12 in the incubator group; in both groups the main indications had been intrauterine growth retardation, or maternal toxaemia, or both (table 1). Four infants in the study group and six in the control group were classified as small for gestational age (below two standard deviations of birth weight, according to the Swedish national growth chart).[3]

There was no significant difference between the mean (SEM) stabilisation periods after birth in the two groups (11 (2) days (range 2–28) in the study group and 15 (4) days (range 3–55) in the control group). Five infants in each group were treated with supplementary oxygen for mild respiratory distress; all recovered without complications. Eleven babies in the study group and 13 in the control group needed phototherapy, but none received an exchange blood transfusion. All the mothers but one in each group produced enough breast milk to feed their infants.

There was no significant difference between the mean (SEM) body weights of the infants in the study group and the control group ((SEM) 1595 (35) g (range 1400–1820) and 1569 (31) g (range 1430–1845)) at the start of the study period.

Table 1 *Characteristics of the study groups at birth*

	Heated, water filled mattress (n=17)	*Incubator (n=17)*	*p Value*
Mean (SEM) birth weight (g)	1567 (52)	1457 (83)	NS
Range	1130–1900	904–1980	
Mean (SEM) length at birth (cm)	41·9 (0·5)	40·1 (0·8)	≤0·005
Mean (SEM) head circumference (cm)	29·0 (0·4)	28·4 (0·6)	NS
Mean (SEM) gestational age (weeks)	32 (0·5)	32 (0·6)	NS
Range	29–35	28–35	
No of babies small for gestational age	4	6	

The heated, water filled mattress (Kanthal Medical Heating AB, Stockholm, Sweden) (fig 1) is a polyvinylchloride bag filled with 10 l water. The mattress is heated by a pad with heating foil covered by plastic and containing five built in thermistors. Two of the thermistors regulate the temperature, two are safety controls to avoid overheating, and one records the temperature of the mattress on a digital display. The heating pad is supplied with 24 V AC with a maximum effect of 50 W. The temperature can be regulated in seven steps between 35° and 38°C, and overheating as well as underheating alarms are built in. When the electricity supply is interrupted the alarm goes off. The mattress is placed in the crib. Each infant is dressed in a cotton shirt, a nappy and a cotton dress. If a baby weighs less than 1500 g he wears a cotton cap. Each infant is placed on the mattress and covered with a blanket.

The incubator used in the study was a single walled, manually controlled, air heated incubator AGA MK41 (AGA AB, Lidingö, Sweden). The infants in the incubators wore only nappies. Temperature adjustments of the mattresses and the incubators were made by the nursing staff after the

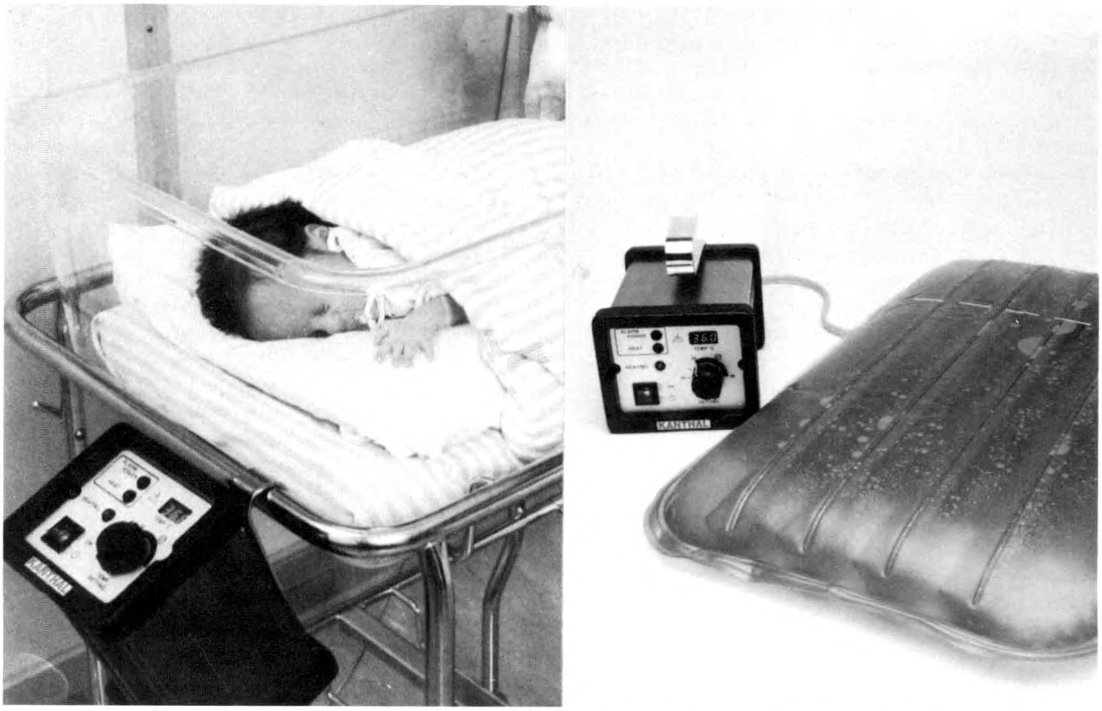

Fig 1 *Heated, water filled mattress. The polyvinylchloride bag is filled with 10 l water. The temperature control unit is connected to the heating pad which is placed under the bag (right). Baby of 1500 g on the mattress, in a crib in the neonatal intensive care unit (left).*

rectal temperatures of the babies had been recorded four times daily with a mercury thermometer. A rectal temperature between 36·5°C to 37·5°C was maintained.[4] The nursery's ambient temperature was maintained at about 25°C (mean (SEM) 24·8 (0·02)°C).

All infants were fed breast milk by nasogastric tube, either with their mother's expressed milk or with a supplement of banked human milk. Feeding volumes were provided according to the recommendations of the European Society of Paediatric Gastroenterology and Nutrition—160 to 220 ml breast milk/kg body weight day after the first postnatal week.[5 6]

The weight was recorded daily to the nearest 5 g and the rate of weight gain was calculated as g/kg body weight/day, and averaged for seven days. Skinfold thickness was measured once a week at five sites (biceps, triceps, subscapularis, flank, and quadriceps), using Harpenden calipers, and the mean skinfold thickness was calculated.[7] Once a week, determinations of heat production and more exact temperature measurements were performed.

The infant's resting oxygen consumption as an indicator of heat production was determined by indirect calorimetry. A continuous open flow circuit method was used with a Plexiglas hood placed over the infant's head. For infants nursed on the mattress the air and wall temperatures of the hood were kept between 24°C and 25°C by circulating water through the inner wall of the hood. The flow of fresh air through the hood was measured with a Fleish pneumotacograph and regulated to achieve a carbon dioxide concentration of 0·5% in the diluted expired air. This resulted in an airflow of 1·0–2·5 l/minute. The pneumotacograph was calibrated against a Tissot spirometer. Air samples from the diluted expired air were drawn every fifth minute during the investigation period. The gas analyses were carried out with a paramagnetic oxygen analyser (Magnos 2T, Switzerland) and an infrared carbon dioxide analyser (Uras 3G, Switzerland). All the volumes were corrected for standard temperature and pressure, dry and computed on line.[8 9] The system was calibrated before and after every investigation with ambient air and a known gas mixture (diluted air with 8% nitrogen and 2% carbon dioxide) using a mixing pump (H Vösthoff OHG, Type Eg725 Bochum, West Germany).

The infant's state of activity was recorded using Brück's method during the heat production measurements.[10] Minimal oxygen consumption was calculated from the values obtained when the baby had been quietly sleeping during at least four periods of five minutes each. Simultaneously temperatures from the following sites were recorded every five minutes, using thermocouples (Exacon 9000, Denmark): rectum (5 cm above the anus); skin (five sites—forehead, anterior abdomen, back, thigh, and foot); incubator air (centrally 10 cm above the infant's body); water in the heated mattress; and room air close to the baby.

The mean skin temperature was then calculated using weighting factors.[11] The accuracy of the thermocouples was checked several times against a 25 cm long mercury thermometer in a water bath at 20° and 40°C, and no deviation was found. The incubator air and the mattress water temperature were adjusted to the same temperature as that previously used.

The mean (SEM) and range were calculated to describe the group characteristics; for the quantitative differences between the groups the unpaired t test (one tail) was used, and for the frequency differences the χ^2 analysis, was used. A p value of $\leq 0{\cdot}05$ was accepted as significant.

Results

The duration of the study varied between infants because of differences in maturation and body weight at the start of the study period. Seventeen, 16, and nine infants in the study group, and 17, 16, and six infants in the control group were studied during one, two, and three weeks, respectively.

The mean weight gain (g/kg body weight/day) was slightly but not significantly higher, during all three of the study weeks in the group treated on the heated, water filled mattress, being 10·8 (2·0) and 8·0 (3·0) during week one, 15·0 (0·6) and 14·0 (0·6) during week two, and 15·5 (1·0) and 13·4 (1·5) during week three.

The mean breast milk feeding volume (ml/kg body weight/day) showed a slight but not significant difference during the first and third study weeks, being 167 (4·1) and 168 (6·5) during week one, 180 (4·1) and 182 (2·8) during week two, and 178 (3·4) and 184 (3·3) during week three. There were no significant differences in the mean skinfold thickness measurements during the study period, with an average gain of 0·4 mm/week for both groups.

Breast feeding was actively encouraged, but the number of mothers who were able to maintain it differed significantly between the groups. At discharge, 11 of 17 babies in the study group were fed only their own mother's breast milk, compared with five of 17 in the control group ($p \leq 0{\cdot}05$). There was no difference in the duration of feeding by nasogastric tube between the two groups.

The results of oxygen consumption are given in table 2. They are given both as the minimal and the average values during the three hour measurement

Table 2 *Mean (SEM) oxygen consumption (ml/kg/minute) in the two groups during the study period*

Week	*Heated, water filled mattress*			*Incubator*			*p Value*
	No of babies	*Minimal consumption*	*Average consumption*	*No of babies*	*Minimal consumption*	*Average consumption*	
1	17	5·9 (0·2)	6·4 (0·2)	17	6·0 (0·1)	6·5 (0·2)	NS
2	16	6·9 (0·2)	7·6 (0·3)	16	6·8 (0·2)	7·6 (0·3)	NS
3	9	7·6 (0·1)	8·0 (0·1)	6	6·8 (0·2)	7·3 (0·1)	≤0·05

period. The minimal oxygen consumption periods occurred approximately two hours after the last meal. No significant difference was found in the minimal and average oxygen consumption during the first two weeks between the two groups. In the third study week, a significantly higher oxygen consumption was observed among babies in the study group ($p\leq 0{\cdot}05$).

The results of the temperature measurements are shown in fig 2. There were no significant differences in the rectal or mean skin temperatures between the groups, but the temperatures on the forehead and foot differed significantly. The mean forehead temperature of the infants in the study group was 1·5°C lower than that of the infants in the incubator group ($p\leq 0{\cdot}001$). On the other hand, the peripheral skin temperature, measured as the foot temperature, was 1·5°C higher in the study group than in the incubator group over the same period ($p\leq 0{\cdot}001$).

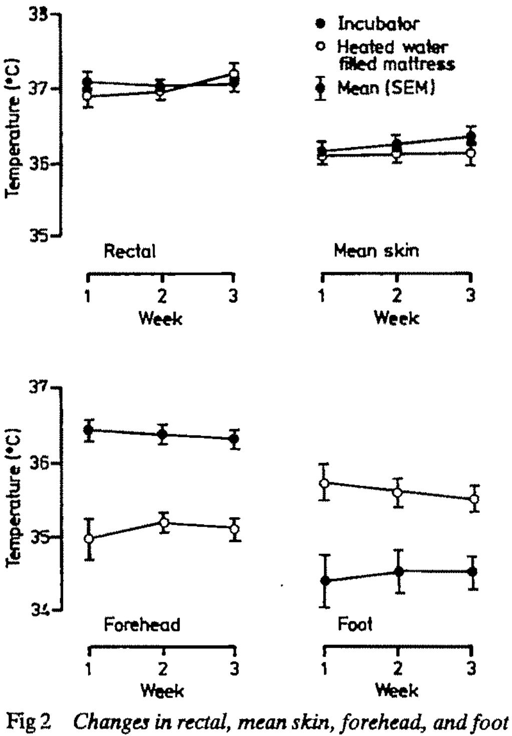

Fig 2 *Changes in rectal, mean skin, forehead, and foot temperatures of babies treated on heated, water filled mattresses and in incubators during the three weeks of the study. During week 1 there were 17 babies in each group, during week 2 there were 16, and during week 3 there were nine and six, respectively;* $^*p<0{\cdot}001$.

The water temperature of the mattress differed from the air temperature in the incubator. The water temperature was close to 36·5°C, with small variation among infants during the three study weeks. On the other hand, the air temperature of the incubator started at 33·5°C and decreased by 0·7°–0·8°C during the study period.

Discussion

The metabolic response of babies nursed in cots was studied in 1970 by Hey and O'Connell, who placed a warming element under an ordinary mattress. The oxygen consumption of 10 babies born at full term weighing 2000 to 3000 g decreased by 22% compared with the heat production recorded in unwarmed babies nursed in cots in an environment of 20°C.[12] The heat production of preterm babies in a cot with a heated, water filled mattress as heat source has not, to our knowledge, been reported. The mattress has several advantages over an ordinary heated mattress, including a high heat capacity, effective heat conduction, and a soft surface.

Our results indicate that the heat received from a heated, water filled mattress is equivalent to that from a conventional incubator. No difference was found in the minimal oxygen consumption, mean skin and body temperatures, and weekly weight gain. Our figures of minimal oxygen consumption in infants on a heated, water filled mattress confirm previous reports about infants in metabolic chambers or incubators.[13 14]

Cot nursing entails exposing the infant's face to the temperature of the ambient room air which, in most nurseries and neonatal wards, varies between 20°C and 25°C. Mestyan *et al* reported that acute cooling of the faces of preterm babies causes a rapid increase in oxygen consumption.[15] These results were not confirmed in our long term investigation. The baby nursed in a cot seems to adapt to the actual thermal conditions when treated continuously on the heated, water filled mattress, and does not increase its oxygen consumption although part of its face is cooled.

One specific advantage of this treatment is that it gives the mother easy access to her child and thus strengthens the early mother infant bonding. This may partly explain the significant increase in breast milk production on discharge from the hospital in the study group.

We conclude that the use of a heated, water filled mattress in a cot seems to be a simple, cheap, effective, and acceptable method of providing heat to healthy preterm babies who do not need intensive care in an incubator. This product can thus be an alternative to the incubator in both developing and developed countries.

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References

[1] Tunell R, Sarman I. The water-filled heated mattress. An alternative to incubator care in developed and developing countries? *Neonatal physiological measurements*. London: Butterworth, 1986;419–23.

[2] Finnström O. Studies on maturity in newborn infants. II. External characteristics. *Acta Paediatr Scand* 1972;**61**:24–32.

[3] Karlberg P, Fryer J, Ericson A, Niklasson A. Analysis of the size at birth by gestational age. *Acta Paediatr Scand* 1985;**319** (suppl):26–34.

[4] Hey EN, Katz G. The optimum thermal environment for naked babies. *Arch Dis Child* 1970;**45**:328–34.

[5] American Academy of Pediatrics Committee on Nutrition. Nutritional needs of low birth weight infants. *Pediatrics* 1985;**75**: 976–86.

[6] ESPGAN Committee on the nutrition of preterm infants. *Acta Paediat Scand* 1987;**336**(suppl):4–5.

[7] McGowan A, Jordan M, MacGregor J. Skinfold thickness in neonates. *Biol Neonate* 1975;**25**:66–84.

[8] Jéquier E. Long-term measurement of energy expenditure in man: direct or indirect calorimetry? *Recent advances in obesity research III. Proceedings of the 3rd International Congress on Obesity*. London: John Libbey, 1981;130–5.

[9] Lister G, Hoffman JI, Rudolph AM. Oxygen uptake in infants and children: a simple method of measurement. *Pediatrics* 1974;**53**:656–61.

[10] Brück K. Temperature regulation in the newborn infant. *Biologia Neonatorum* 1961;**3**:65–119.

[11] Klein AD, Scammon RE. The regional growth in surface area of the human body in prenatal life. *Proc Soc Exp Biol Med* 1930;**27**:463–6.

[12] Hey EN, O'Connell B. Oxygen consumption and heat balance in the cot-nursed baby. *Arch Dis Child* 1970;**45**:335–43.

[13] Scopes JW, Ahmed I. Minimal rates of oxygen consumption in sick and premature infants. *Arch Dis Child* 1966;**41**:407–16.

[14] Sinclair J, Scopes J, Silverman W. Metabolic reference standards for the neonate. *Pediatrics* 1967;**39**:724–32.

[15] Mestyan J, Jarai I, Bata G, Fekete M. The significance of facial skin temperature in the chemical heat regulation of premature infants. *Biol Neonate* 1964;**7**:243–54.

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Routine endotracheal cultures for the prediction of sepsis in ventilated babies

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SUMMARY Serial cultures of endotracheal tube aspirates were carried out in 94 neonates who were intubated and had been ventilated for longer than one week. A similar change in bacterial colonisation with duration of ventilation was seen in infants who subsequently developed sepsis and those who did not. In both groups, 177 aspirates (more than 90%) obtained during the first week of ventilation were sterile. Thereafter, colonisation with mixed Gram positive flora emerged followed by growth of Gram negative bacilli. For the infants who developed sepsis, the correlation between pathogens isolated from blood during the episodes of sepsis and those from previous endotracheal tube isolates was poor; in only five of the 26 cases of sepsis (19%) was the same single strain of organism isolated from culture of the blood and of the endotracheal tube aspirate. Other markers of infection such as endotracheal tube aspirate white blood cell counts and changes in bacterial flora were not useful in predicting systemic infection. Routine surveillance cultures of endotracheal tube aspirates in ventilated infants are not helpful in predicting the pathogens that are isolated from the blood during episodes of sepsis.

Hospital acquired infection is an important cause of mortality and morbidity among infants in neonatal intensive care units. Surveillance cultures are performed routinely in many units to monitor infants at risk of sepsis.[1 2] For example, cultures of aspirates obtained through endotracheal tubes from ventilated infants are monitored on the assumption that the organisms that are colonising the respiratory tract will be the same pathogens as those isolated from the blood during episodes of sepsis. Support for this practice comes from studies showing that in infants who develop sepsis at birth, the same bacterial pathogens can be recovered from cultures of blood and endotracheal tube aspirates.[3–5] The value of serial cultures of endotracheal tube aspirates, however, in determining the aetiology of sepsis in infants who remain intubated for longer periods of time has not been proved. Furthermore, the pattern of organisms colonising the endotracheal tube in ventilated infants after the first week of life has not been elucidated.

This study was designed to define the pattern of respiratory tract colonisation in neonates intubated and ventilated for longer than one week and to assess the value of serial cultures of endotracheal tube aspirates in predicting pathogens isolated during episodes of sepsis.

Patients and methods

The study population was selected from the 657 neonates born between 1 January 1985 and 31 December 1985 who were admitted to the neonatal intensive care unit at Crouse Irving Memorial Hospital. Ninety four of the 657 neonates (14%) required endotracheal intubation and mechanical ventilation for longer than one week and were included in the study. Tracheal aspirates were collected through endotracheal tubes into DeLee traps under sterile conditions every three days for as long as infants remained ventilated.[6] A Gram stain was performed on all aspirates for the identification of bacteria and measurement of the white blood cell count. White cells were counted from 20 high power fields and the average taken. All aspirates were plated immediately onto blood, chocolate, and MacConkey agars. Plates were examined daily and organisms identified by routine laboratory techniques.[7 8] Antibiotic sensitivity testing was done on all Gram negative bacilli and coagulase positive staphylococci that were isolated. Infants suspected of having a septic focus on the basis of clinical findings had blood from at least one percutaneous puncture site inoculated into tryptic soy broth. Antibiotic sensitivity testing was done on all organisms isolated from blood cultures. Over the year of study, all infants requiring ventilation received antibiotics for the first three days of life. Thereafter, antibiotics were given only during episodes of suspected or proved sepsis.

The pattern of respiratory tract colonisation over time in infants who developed sepsis was compared with that of infants who did not. Additionally, for infants who developed sepsis, correlation between blood pathogens and isolates obtained from endotracheal tubes during the week before the episode of sepsis was examined. The following definitions were used: full agreement when the organism strain isolated from blood matched the only isolate from the endotracheal tube; partial agreement when the organism strain isolated from the blood matched one of several isolates from the endotracheal tube; and no agreement when the organism found in the blood did not match any of those isolated from the endotracheal tube.

Differences in the characteristics between infants with and without sepsis were evaluated by the two tailed Student's *t* test. Differences in the Gram stain white cell response were compared by Fisher's exact probability test. All values are expressed as mean (SEM).

Results

The study group comprised 54 boys and 40 girls who required endotracheal intubation and mechanical ventilation for a mean of 47 (5) days (range 8 to 276). All but three infants were intubated and ventilated on the first day of life. The most common reason for ventilation was respiratory distress syndrome, which occurred in 63 (67%) of the infants. During the study period 26 episodes of sepsis occurred in 22 of the ventilated infants. These 22 infants with sepsis differed from the remaining 72 ventilated infants who never developed sepsis in that they weighed less at birth (926 (116) compared with 1410 (118) g, $p<0\cdot01$), were of lower gestational age (27·5 (0·7) compared with 29·6 (0·6) weeks, $p<0\cdot05$), and required mechanical ventilation for a longer period of time (83 (15) compared with 36 (4) days, $p<0\cdot001$). The mean (SEM) age of infants when bacteraemia was diagnosed was 41 (7) days, range 8–130.

A total of 1448 cultures of endotracheal tube aspirates were done on the entire group of 94 infants; 395 (27%) grew no organisms, 410 (28%) grew mixed Gram positive flora (including coagulase negative staphylococci, α haemolytic streptococci, and diphtheroids) and the remaining 643 cultures (45%) grew one or more Gram negative bacilli, coagulase positive staphylococci, or other pathogens.

Bacterial colonisation of endotracheal tube aspirates changed similarly with the duration of ventilation in infants who developed sepsis and in those who did not (fig 1). In both groups of infants, more than 90% of the aspirates (177) obtained during the first week of ventilation were sterile. Between the second and fourth weeks of ventilation, respiratory colonisation with mixed Gram positive flora developed, and after four weeks of ventilation Gram negative bacilli predominated. *Klebsiella pneumoniae*, *Pseudomonas* species, *Enterobacter* species, and *Escherichia coli* were not isolated

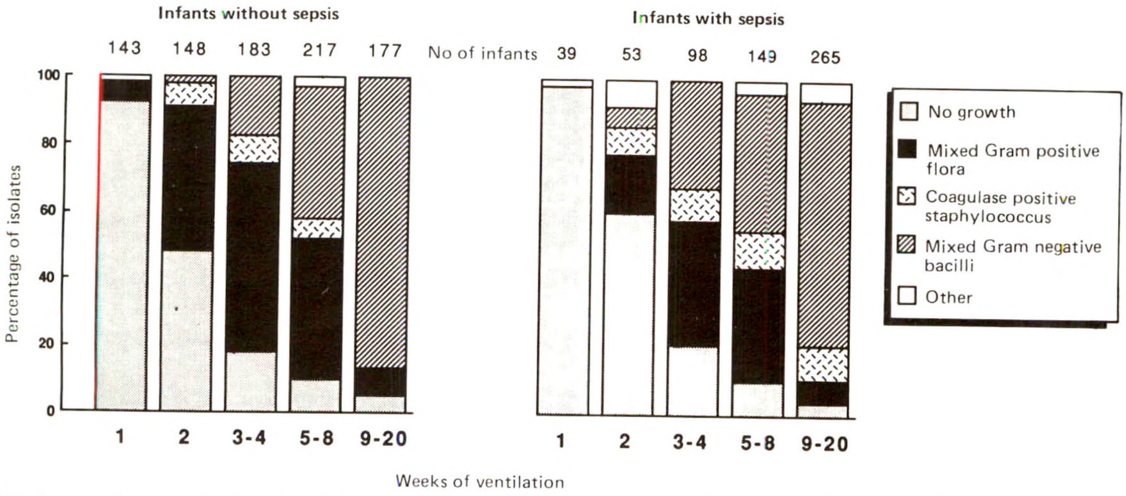

Fig 1 *Developmental pattern of bacterial colonisation of endotracheal tube aspirates in infants with and without sepsis.*

during the first week of ventilation but accounted for 138 (almost 40%) of all isolates obtained during the second month of ventilation and 316 (over 70%) of all isolates obtained after two months of ventilation.

In the infants with sepsis, pathogens isolated from blood were compared with endotracheal tube isolates obtained during the week before the onset of clinical infection (fig 2). In 14 of the 26 cases of sepsis (54%), the strain of organism isolated from blood was not found in the endotracheal tube aspirate. These cases of 'no agreement' included five instances in which the previous endotracheal tube aspirates had been sterile and nine instances in which

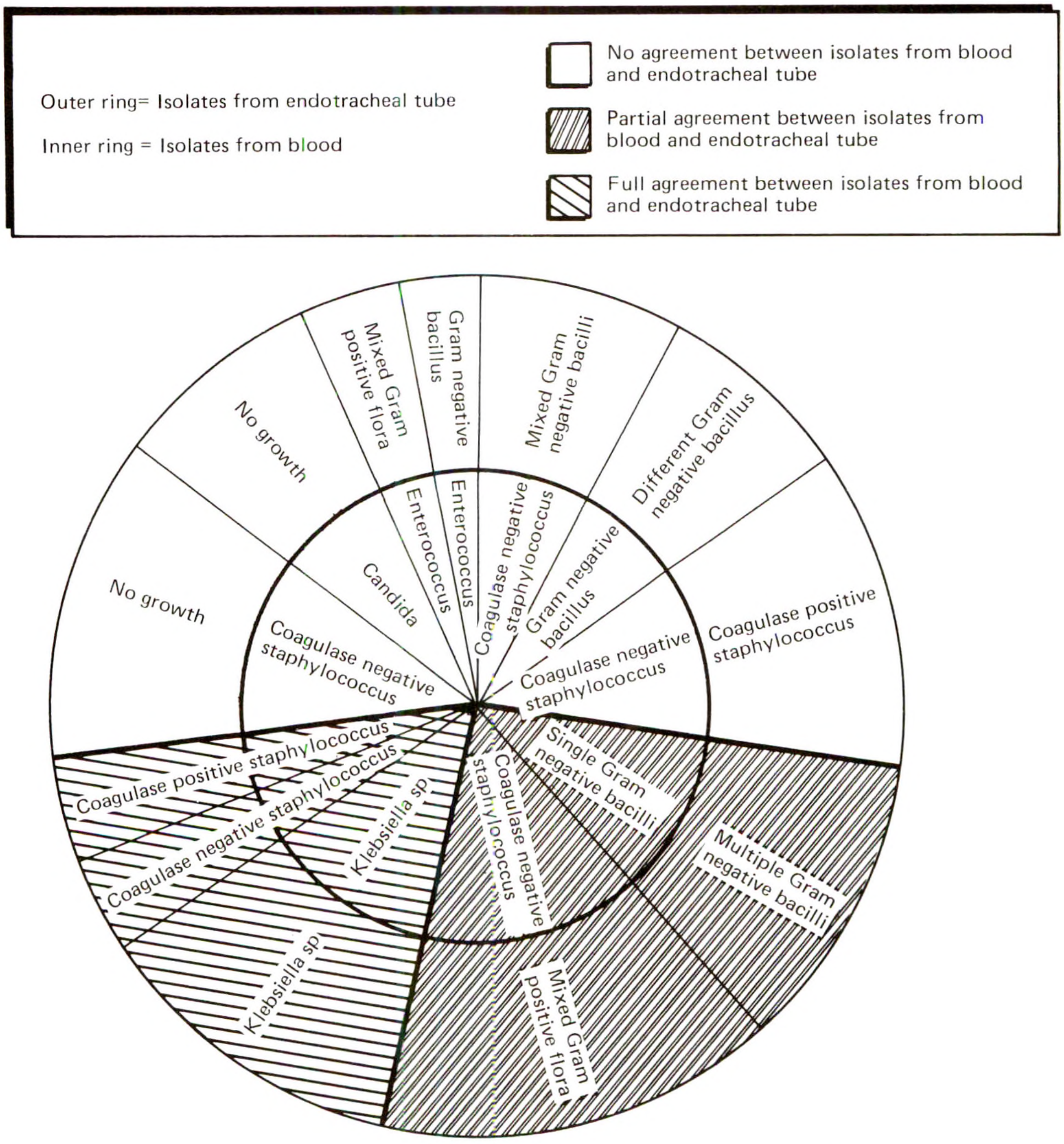

Fig 2 *Agreement between blood pathogens isolated during episodes of sepsis and bacteria isolated from endotracheal tube aspirates during the previous week.*

different organisms were isolated from the endotracheal tube aspirates. In seven of the 26 cases of sepsis (27%), the organism in the blood was found as one of multiple isolates from the endotracheal tube (partial agreement). Finally, in only five cases of sepsis (19%) was the same single strain of organism isolated from both blood and endotracheal tube aspirate cultures (full agreement).

The length of time before an episode of sepsis that any single organism was grown from the endotracheal tube aspirate was compared in the three agreement groups. The infants with full agreement and those with partial agreement had their blood pathogen identified in endotracheal tube aspirates for a mean 17 (3) days and 11 (5) days before the onset of sepsis, respectively. These durations of endotracheal tube colonisation with a single organism before the diagnosis of sepsis did not differ significantly from that seen in infants with no correlation between blood and endotracheal tube isolates (10 (3) days).

The organisms responsible for sepsis in the ventilated infants were similar to organisms isolated from blood in 23 non-ventilated infants who developed sepsis after 1 week of age during the one year study period. Coagulase negative staphylococci were the most frequent pathogens found in the blood in both groups, accounting for 50% of positive blood cultures in ventilated infants and 11 (48%) of those in non-ventilated infants. *Klebsiella* was the next most common pathogen and was responsible for 23% and 17% of cases of sepsis in ventilated and non-ventilated infants, respectively.

The endotracheal tube aspirate white blood cell response seen in infants during the week before an episode of sepsis was similar to that seen in aspirates taken from infants who never developed sepsis. Only four of 73 aspirates (5%) obtained from infants who developed sepsis had more than 10 white cells/high power field while 74 of 818 aspirates (9%) obtained from infants who never developed sepsis had a similar response. None of the Gram stains of aspirates taken from infants with full agreement between blood and endotracheal tube pathogens had this degree of white cell response.

Finally, if antibiotic choices had been based on the sensitivity of the predominant isolate from the culture of the endotracheal tube aspirates obtained just before the manifestations of sepsis there would have been inappropriate antibiotic coverage for the pathogen in the blood in 11 of the 26 cases (42%).

Discussion

Bacterial colonisation of the respiratory tract changed with time in neonates requiring prolonged endotracheal intubation and mechanical ventilation. Of the endotracheal tube aspirate cultures obtained from infants during the first seven days of ventilation, 90% were sterile. The lack of organisms was probably the result of administration of antibiotics to all infants during the first three days of life. Harris *et al*[3] found a significant decrease in respiratory tract colonisation in the first several days of life in ventilated infants treated with antibiotics compared with similar infants who received no antibiotics. In our population, after the first week of ventilation growths of mixed Gram positive flora emerged, and after a month of ventilation growths of Gram negative bacilli predominated. This pattern of respiratory tract colonisation was similar in infants who subsequently developed sepsis and in those who remained asymptomatic.

The value of the body surface colonisation pattern as a predictor of pathogens responsible for episodes of neonatal sepsis has been questioned. Evans *et al*[2] obtained cultures from 3371 infants admitted to a single newborn intensive care unit to determine how frequently isolates from body fluid cultures, primarily blood, matched those seen on previous body surface cultures. A total of 1935 infants had at least one surveillance culture of skin, mucous membrane, or gastric or endotracheal tube aspirate done during the two week period before an episode of suspected sepsis. Although agreement between the results of cultures of body fluid and surface isolates improved the nearer the time the body surface cultures were obtained to an episode of proved sepsis, the maximal sensitivity of all surface cultures was only 56% while overall specificity was 82%. When the surveillance cultures of endotracheal tube aspirates were analysed separately, the sensitivity and specificity were 26% and 74%, respectively. These investigators studied a heterogeneous group of infants, and results of surface cultures reflected isolates acquired from both perinatal and nosocomial exposure. Our study was designed to evaluate whether identifying respiratory tract colonisation aided the management of ventilated neonates at high risk of hospital acquired sepsis.

Previous studies examining the association between respiratory tract colonisation and systemic infection have included both non-ventilated infants and infants ventilated for brief periods of time. Sprunt *et al*[9] prospectively monitored oropharyngeal colonisation in 223 nursery admissions between 1971 and 1974. In that study none of the 156 infants colonised with α haemolytic streptococci or other bacteria in low titre developed sepsis. In contrast, 14 of 67 infants colonised with potential pathogens in high titre developed systemic infection. In almost every case these infections were caused by colonising bacteria. The effects of endotracheal intubation and mechanical ventilation on respiratory colonisation or infection were not evaluated.

Harris *et al*[3] prospectively studied 54 neonates who required orotracheal intubation. Respiratory tract colonisation was monitored with nasopharyngeal as well as tracheal aspirate cultures. The respiratory tracts of 23 infants were already colonised at the time of intubation; nine of the infants (39%) were concurrently infected. Sixteen additional infants subsequently developed respiratory tract colonisation and eight of those (50%) developed sepsis. In contrast, none of the 10 infants who remained uncolonised developed sepsis. As more than 80% of all infants were intubated for less than three days the correlation between respiratory tract flora and organisms isolated from blood may have reflected congenital infections. Sherman *et al*[4 10] provided data showing that tracheal aspirates obtained within the first eight hours after birth were reliable in establishing the diagnosis of congenital pneumonia and bacteraemia. The authors pointed out, however, that the presence of bacteria in tracheal secretions lost its diagnostic usefulness after the infant was more than 24 hours old when the normally sterile fetal lung becomes colonised. Most ventilated infants in our series who developed sepsis after one week of life showed no correlation between pathogens isolated from blood and previous endotracheal tube isolates. Other putative markers for infection such as endotracheal tube aspirate white cell response and changes in respiratory tract bacterial flora were also unhelpful in identifying infants who subsequently developed sepsis. The part played by serial cultures of endotracheal tube aspirates in the diagnosis of hospital acquired infections that are limited to the respiratory tract (such as tracheitis and pneumonia) remains to be elucidated.

The use of routine surveillance cultures other than those directed at tracing a specific organism during an epidemic[11] has been likened to an exercise in futility.[12] Though serial cultures of endotracheal tube aspirates may be useful in establishing the general nursery flora, their limited value to an individual infant does not justify routine surveillance in all ventilated infants.

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References

[1] Sprunt K. Practical use of surveillance for prevention of nosocomial infection. *Semin Perinatol* 1985;**9**:47–50.

[2] Evans ME, Schaffner W, Federspiel CF, Cotton RB, McKee KT, Stratton CW. Sensitivity, specificity, and predictive value of body surface cultures in a neonatal intensive care unit. *JAMA* 1988;**259**:248–52.

[3] Harris H, Wirtschafter D, Cassady G. Endotracheal intubation and its relationship to bacterial colonization and systemic infection of newborn infants. *Pediatrics* 1976;**56**:816–23.

[4] Sherman MP, Goetzman BW, Ahlfors CE, Wennberg RP. Tracheal aspiration and its clinical correlates in the diagnosis of congenital pneumonia. *Pediatrics* 1980;**65**:258–63.

[5] Brook I, Martin WJ, Finegold SM. Bacteriology of tracheal aspirates in intubated newborn. *Chest* 1980;**78**:875–7.

[6] Gregory GA. Respiratory care of newborn infants. *Pediatr Clin North Am* 1971;**19**:311–24.

[7] Lennette EH, Balows A, Hausler WJ, *et al. Manual of clinical microbiology.* Washington: American Society for Microbiology, 1985.

[8] Isenberg HD, Schoenknecht FD, von Graevenitz A. *Cumulative techniques: collection and processing of bacteriologic specimens.* Washington: American Society for Microbiology, 1979.

[9] Sprunt K, Leidy G, Redman W. Abnormal colonization of neonates in an intensive care unit: means of identifying neonates at risk of infection. *Pediatr Res* 1978;**12**:998–1002.

[10] Sherman MP, Chance KH, Goetzman BW. Gram's stains of tracheal secretions predict neonatal bacteremia. *Am J Dis Child* 1984;**138**:848–50.

[11] Smith DH. Epidemics of infectious diseases in newborn nurseries. *Clin Obstet Gynecol* 1979;**22**:409–23.

[12] Fulginiti VA. Body surface cultures in the newborn infant. An exercise in futility, wastefulness and inappropriate practice. *Am J Dis Child,* 1988,**142**:19–20.

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Prediction and diagnosis of sickling disorders in neonates

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SUMMARY A pilot scheme for the prediction and detection of sickle cell disease in neonates was set up and the results from its first three years of operation analysed. A total of 153 women booking at the antenatal clinic were found to have haemoglobin S. The protocol required that all partners of the women so identified be screened for abnormal haemoglobins and β thalassaemia trait, and that the babies of these women should have cord blood electrophoresis performed. In fact this was only achieved in 75 partners (49%) and 91 of 145 infants (63%). Of 10 babies born with sickle cell disease during the study period, nine had mothers known to have haemoglobin S or C, but only four of these mothers' partners had been tested before delivery of the child.

This pilot study highlights the problems in establishing effective antenatal screening programmes, especially in ethnic minority groups. The results show that detailed staff training, careful organisation of administrative arrangements, and education of 'at risk' groups should all be undertaken before the launching of major screening initiatives.

The mortality from sickle cell disease is high in the first two years of life.[1 2] It has been suggested that mortality is lowered by early detection as this ensures that medical and child health staff are alerted to potential problems and it permits the education of parents and the administration of prophylactic penicillin to the infant.[3–5]

Paddington and North Kensington district is a deprived multiethnic inner city health district where 10% of births are to mothers of African or Caribbean origin. In 1983 a multidisciplinary team drawn from the departments of obstetrics, paediatrics, child health, and haematology was convened to study the problems associated with the establishment of a screening programme for the prediction and detection of sickling disorders in the neonate. A protocol was drawn up so that all women were managed according to the same guidelines. This paper presents the results of the first three years of the study (1 July 1983 to 30 June 1986). As is conventional, we have used the term 'sickle cell anaemia' to indicate homozygosity for the sickle cell gene and 'sickle cell disease' to include also other sickling disorders such as double heterozygosity for both the sickle cell gene and either β thalassaemia trait or haemoglobin C. A programme to predict β thalassaemia major in neonates was introduced simultaneously with the sickle cell screening programme.[6]

Patients and methods

All women booking at district antenatal clinics had their ethnic origin recorded in a designated box on haematology request forms using the following general categories: black, Indian subcontinent, oriental, Mediterranean, caucasian (other than Indian subcontinent and Mediterranean), and other. Antenatal clinic staff requested a full blood count, blood film examination, and sickle solubility test at booking on all women classified as black. If the antenatal clinic staff identified a woman as black but failed to request a sickle screen the protocol required that the test be initiated by laboratory staff. If the sickle solubility test was positive, haemoglobin electrophoresis was performed. In addition, black women with microcytic red cells (mean corpuscular volume less than 83 fl) had haemoglobin electrophoresis and a haemoglobin A_2 estimation performed as part of the programme for prediction of β thalassaemia major.[6]

In the early part of the study it was not considered feasible to screen all black women for the presence of haemoglobin C, but a small number of women who were sickle negative had haemoglobin electrophoresis performed because target cells were noted in the blood film. In the latter part of the study haemoglobin electrophoresis was performed on the blood of all black women irrespective of the results of the sickle solubility test. Because no extra staff or other resources were available to establish a formal screening programme, a decision was made to restrict screening for haemoglobin S to black women rather than also to attempt systematic screening of other ethnic groups where a lower but appreciable incidence of the sickle cell gene occurs. The diagnosis of sickle cell trait was therefore made only occasionally in non-black women. It was because of the same constraints that we did not initially routinely screen for haemoglobin C.

Women presenting before 24 weeks of gestation in whom a diagnosis of sickle cell trait, haemoglobin C trait, or β thalassaemia trait was made were contacted by telephone, in the first instance by the antenatal clinic sister who gave a brief explanation of the test result and invited the woman to bring her partner to the clinic for testing. The woman and her partner were then given a further explanation of the test result by a doctor of at least registrar status. Tests performed on the blood of partners were full blood count, sickle solubility test, haemoglobin electrophoresis (irrespective of the results of the sickle solubility test) and, if the partner's red cells were microcytic, haemoglobin A_2 estimation. When tests in the partner indicated a risk of sickle cell disease in the fetus and the patient was not already seeing the consultant, referral was arranged, except in those instances when the patient stated that she did not have any interest in antenatal diagnosis. The patient was advised as to the possible outcome of pregnancy, and when sufficiently early in pregnancy, the options of antenatal diagnosis and termination of pregnancy were fully discussed. Referral to a Sickle Cell Centre was offered when women wished to have the opportunity for further explanation and discussion. Patients who presented later than 24 weeks of gestation were in general not recalled early but an explanation was given and testing of the partner was offered at the next clinic attendance.

The protocol required that cord blood samples be obtained from all black babies whose mothers were known to have sickle cell trait, haemoglobin C trait, or β thalassaemia trait. The labour ward was alerted to the need for such tests by (a) a statement of the sickle status on the front of the obstetric notes and (b) either a stamp or a written request on the labour ward page. Cord blood samples were also required on black babies whose mothers presented for the first time already in labour, or whose haemoglobin status was otherwise unknown. Cord blood samples were anticoagulated with edetic acid and were used for a full blood count and haemoglobin electrophoresis. When a cord blood sample showed a baby to have sickle cell disease the baby and the mother were recalled to a joint haematology and paediatric clinic. When cord blood showed haemoglobin F only or haemoglobins F, S, and A with haemoglobin S apparently in excess of haemoglobin A (possible haemoglobin S/β^+ thalassaemia) the mother and the baby were recalled at six weeks for further testing. Recalling of mothers and babies was performed by a lecturer in community paediatrics.

In order to check as far as possible whether all cases of sickle cell disease in neonates had been predicted by the programme, we examined the records of all cord blood electrophoresis performed for any reason and all electrophoresis on infants performed within the district during the period of the study and for seven months thereafter.

Sickle solubility tests were performed using the Mercia Diagnostics S-test kit. Haemoglobin electrophoresis was performed on cellulose acetate at pH 8·2 to 8·6. When necessary (to distinguish haemoglobins D and G from S and to distinguish between haemoglobins C and E) electrophoresis was also performed on citrate agar at pH 6·0 to 6·2.

At the beginning of the study period information sheets for both staff and pregnant women were drawn up and distributed to the antenatal clinics and to all senior medical and midwifery staff. Appropriate seminars and study days were arranged.

Results

During the three years surveyed, 153 women were identified as having haemoglobin S; 149 had sickle trait, two were double heterozygotes for S and C, one had sickle cell anaemia, and one was heterozygous for both haemoglobin S and the α chain variant haemoglobin G Philadelphia. A total of 148 women who were sickle positive were black, three were Indian, one Moroccan, and one Libyan. Of the five non-black women, four were investigated because they were microcytic and one because haemoglobin electrophoresis was specifically requested by obstetric staff.

In 57 cases (37%) the woman's partner was investigated by us and in a further 18 cases (12%) the woman stated that her partner had already been investigated and was known to be sickle negative; partners were therefore investigated in 75 instances (49%). When partners were not investigated explanations were sometimes recorded in the notes as follows: 12 women miscarried or transferred to another clinic; in 14 instances testing was offered but was not desired; in seven cases the partner was not tested because he was caucasian; in five instances the partner was not in the United Kingdom and in six cases he was otherwise unavailable. In 34 cases (22%) no reason for not testing the partner was recorded in the notes; this group included many women who presented late in pregnancy or already in labour.

A total of 141 women gave birth to a total of 145 live infants. Of the 145 infants, cord blood samples were obtained in 91 instances (63%). The results of electrophoresis of these cord blood samples are shown in the table.

A definitive diagnosis of sickle cell disease was made in five cases (5·5% of cord bloods screened); one case was of sickle cell/haemoglobin C disease, and four were either sickle cell anaemia or double heterozygosity for haemoglobin S and β° thalassaemia. In 12 instances (13·3%) further follow up electrophoresis was required because only haemoglobin F was present at birth or other bands were too faint to allow a confident diagnosis. Such follow up proved difficult: in five instances the infant was retested by us or elsewhere but in seven cases efforts to have the baby brought to the clinic for repeat testing were unsuccessful.

Investigation of the results of haemoglobin electrophoresis performed on cord bloods and on the blood of infants during and after the period of this study disclosed a total of 10 instances of sickle cell disease in the infants of mothers who became pregnant during the study, three of the 10 being double heterozygotes for haemoglobin S and haemoglobin C. Because of the method of ascertainment 10 cases may well be an underestimate. Mothers of six of the 10 cases were detected by the screening programme, five being the babies of mothers with haemoglobin S; the sixth was the baby of a mother whose antenatal screening had shown her to have haemoglobin C trait. Another three babies were born in hospital but their mothers had never attended the antenatal clinic; all antenatal care including sickle screening had been provided by their general practitioner. In the one remaining instance the mother had attended the hospital antenatal clinic but had not been tested for haemoglobin S; in this patient both the antenatal clinic staff and the laboratory staff failed to initiate the test and no further action was taken by antenatal staff on subsequent clinic attendances. Of the nine mothers who had been tested antenatally one stated that she was not aware of the test result. Only four fathers had been tested before the delivery of the child; in three cases the test had been offered and declined but in three cases there was no record that the test had been offered. In addition one mother said that she had been unaware of the test result in her partner. One couple had been offered antenatal diagnosis and had accepted but had decided to continue with the pregnancy after the diagnosis of sickle cell anaemia as the mother had felt fetal movements and this made termination of pregnancy unacceptable to her.[7]

The extension of the use of haemoglobin electrophoresis to all black women, irrespective of the results of the sickle solubility test, did not disclose any unexpected cases of sickle cell trait, although the rate of diagnosis of haemoglobin C trait increased considerably.

Discussion

Many of the problems involved in predicting the possibility of neonatal sickle cell disease and in offering antenatal diagnosis are highlighted in this study. Many women in the groups at risk present late in pregnancy. Sometimes ethnic origin is difficult to categorise, and not all women are aware of

Table *Results of cord blood haemoglobin electrophoresis (n=91)*

Haemoglobins detected	*Interpretation*	*No of infants*	*Follow up*
F+A	Normal	43	—
F+A+S	Sickle cell trait	28	—
F+S	Probable sickle cell disease (sickle cell anaemia or S/β° thalassaemia)	6	4 sickle cell anaemia or S/β° thalassaemia, 1 sickle trait, 1 failed follow up
F+S+C	Heterozygosity for haemoglobins S and C (sickle cell/haemoglobin C disease)	1	1 sickle cell/haemoglobin C disease
F only or F+indistinct bands	Follow up required for interpretation	12	4 sickle trait, 1 referred elsewhere, 7 not traced
Other	Haemoglobin C trait or other abnormal haemoglobins	1	1 Haemoglobin C trait

their racial ancestry. Some antenatal clinic staff are reluctant to categorise women by race, fearing that their motives may be misunderstood, and some simply forget to do it. Blood samples sometimes get lost and some results never reach the clinic or arrive after a long delay. The importance of a positive result may not be appreciated, particularly by staff new to the area, or staff may forget to act upon it. Some women do not wish to have their partners tested, and partners are sometimes unwilling to be tested or unavailable. In our series fewer than half the partners were investigated. Counselling about antenatal diagnosis and possible termination of pregnancy is also a difficult area. The severity of sickle cell disease is unpredictable and many medical and nursing staff have ambivalent feelings as to whether or not it is justified to offer termination of pregnancy. Although most feel that parents have a right to know that they may produce a child with a serious disorder and that termination of affected pregnancies is available, they may unwittingly communicate their ambivalence to the parents. In our experience the number of parents who choose to attend for more detailed counselling varies with the enthusiasm of the medical and nursing staff. We think it is important that professional counselling is available, preferably from those with direct experience of sickle disease, and it often helps if the counsellors are from the same ethnic background (such as, for example, those provided by the Sickle Cell Society).

Some of these problems might be avoided by performing electrophoresis on all antenatal women. As most hospitals will have to conduct screening without any increase in funding, however, such a policy is not likely to be affordable; it certainly was not in our case. We did screen for the β thalassaemia trait which may interact with haemoglobin S from the partner; the results of this programme are reported elsewhere.[6] We recognised that haemoglobin C might escape detection but as sickle cell/haemoglobin C disease is less severe than sickle cell anaemia we felt this was acceptable at the time; we have now expanded our screening to detect haemoglobin C also.

Our study has also showed some of the problems that occur in screening neonates for sickle cell disease. It has been suggested that in certain communities all neonates should be screened.[3 5] One such programme in a community in which 3% of babies were black was abandoned after screening 7691 babies because no significant haemoglobinopathies had been found.[8] In two other communities with 10%[9] and 22%[5] of babies being black the cost of each case detected was estimated at £705 and £118 respectively (about £885 and £135 at current prices). Because of resource constraints we chose to screen only babies of black mothers who had not been tested and of those known to have haemoglobin S or C or β thalassaemia trait. A selective programme such as this may mean that some affected babies are not detected.

We succeeded in testing only 63% of babies whose mothers had haemoglobin S. This was due both to failure to flag the notes appropriately in the antenatal clinic, and to labour ward staff overlooking the request for cord blood testing stamped on the labour ward page. A policy of testing all black babies or all non-caucasian babies would be administratively simpler than highly selective screening and might lead to testing of a higher percentage of 'at risk' babies. It may be that in communities with a high percentage of non-caucasians comprehensive screening programmes will be found to be necessary if early deaths in infants with sickle cell disease are to be prevented. In our region plans are underway to perform electrophoresis on all samples of blood taken by heel prick on the sixth day of life for Guthrie testing. Careful monitoring of the cost and efficacy will be necessary to ensure that the approach is justified; such monitoring should also be performed in any hospital setting up an antenatal screening programme.

Even when testing of cord blood or of a sample from a neonate shows an abnormality, problems exist in adequate follow up. We were unable to carry out further necessary testing in eight instances. This was consequent on mothers not being traceable or not responding to repeated requests to bring the baby to the clinic. Similar problems in ensuring adequate follow up have been noted in the United States.[4] Adequate follow up of 'at risk' babies requires an effective mechanism of notification from the laboratory to child health workers and requires a team member who can, when necessary, trace mothers and do home visits. Unless such support is available then resources devoted to the diagnosis of sickle cell disease in the neonate may be wasted.

The findings of our study provide useful information on the difficulties that will be encountered by hospitals seeking to establish a diagnostic service. In the light of this pilot study, we feel there are three important areas that should be addressed before any screening service is set up. Firstly, staff must be thoroughly educated. We found a suprising level of ignorance among both medical and midwifery staff, at junior and senior level. Secondly, administrative arrangements for notification of abnormal results, recall, and counselling of patients need to be planned carefully. As a result of our findings during the pilot study, we have now introduced more formalised arrangements for ensuring recall and to check that counselling has in fact been provided. Thirdly, a major factor in the surprisingly low uptake of testing was the low level of awareness of the problem in the black population. We consider that educational programmes need to be disseminated much more widely in the 'at risk' populations. This does raise problems of perception in that screening programmes are sometimes seen as racist; however, we anticipate that the offering of screening programmes for cystic fibrosis to the white population, which is expected to occur soon, will help to make this a less contentious issue.

Despite the problems, we consider that antenatal screening for sickle cell disease is an important service that should become generally available.

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References

[1] Rogers DW, Clarke JM, Cupidore L, Ramlal AM, Sparke BR. Early deaths in Jamaican children with sickle cell disease. *Br Med J* 1978;i:1515–6.

[2] Warren NS, Carter TP, Humbert JR, Rowley PT. Newborn screening for hemoglobinopathies in New York State: experience of physicians and parents of affected children. *J Pediatr* 1982;**100**:373–7.

[3] John AB, Ramlal A, Jackson H, Maude GH, Sharma AW, Serjeant GR. Prevention of pneumococcal infection in children with homozygous sickle cell disease. *Br Med J* 1984;**288**:1567–70.

[4] Grover R, Shahidi S, Fisher B, Goldberg D, Wethers D. Current sickle cell screening for newborns in New York City 1979–1980. *Am J Public Health* 1983;**73**:249–52.

[5] Henthorn J, Anionwu E, Brozovic M. Screening cord blood for sickle cell haemoglobinopathies in Brent. *Br Med J* 1984;**289**:479–80.

[6] Bain BJ. Screening of antenatal patients in a multiethnic community for β thalassaemia trait. *J Clin Pathol* 1988;**41**:481–5.

[7] Horwell DH, Heaton DE, Old JM. Diagnosis of haemoglobinopathy using cultured chorionic villus cells. *Lancet* 1985;ii:613–4.

[8] Evans DIK, Blair VM. Neonatal screening for haemoglobinopathy. Results in 7691 Manchester newborns. *Arch Dis Child* 1976;**51**:127–30.

[9] Griffiths KD, Raine DN, Mann JR. Neonatal screening for sickle haemoglobinopathies in Birmingham. *Br Med J* 1982;**284**:933–5.

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Short reports

Suckling stimulation test for neonatal tremor

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SUMMARY The response of neonatal tremor to a suckling stimulation test was investigated in 102 healthy neonates born at full term. In 84 the tremor resolved immediately; none had hypocalcaemia and only one had mild hypoglycemia. Eighteen in whom the tremor continued had either hypocalcaemia (n=13) or hypoglycaemia (n=5).

Neonatal tremor is common, and has various causes including neurological damage and some biochemical deficiencies. These infants are usually otherwise normal on physical examination, and the batteries of tests to which they are often subjected cause much distress to the infant and to the parents. A review of published reports failed to disclose a simple method for differentiating between physiological and pathological tremor. This study was designed to test the hypothesis that cessation of the tremor by the suckling stimulation test indicates a physiological tremor.

Patients and methods

Of 9408 newborn infants born at the Hadassah Medical Centre between August 1984 and February 1988, 143 were found to have neonatal tremor at the routine physical examination carried out during the first 72 hours of life and before the morning meal. Neonatal tremor was defined as repetitive movements of both hands (with or without movement of the legs or jaws) at a frequency of 2–5/s and lasting more than 10 minutes. A total of 102 infants who fulfilled the following criteria were included in the study: gestational age ≥37 weeks; birth weight ≥2500 g; Apgar score ≥8 at five minutes, and a physical examination that was otherwise normal. Forty one neonates were excluded from the study because they were premature, or small for gestational age, or had diabetic mothers, or had other abnormalities on physical examination. Twenty five healthy babies born at full term without tremor were selected sequentially after each infant enrolled in the study to serve as a reference group. Characteristics of the patients and the reference group are shown in table 1.

Infants were examined on the first and third day of life at a mean (SD) of 30 (17) minutes before their morning meal (range 6–66 minutes). The suckling stimulation test consisted of the examiner putting his middle finger into the mouth of the infant who was lying supine with the head straight and both hands free. Each test was performed twice, the second time by another examiner. A test was positive if the tremor stopped instantly and returned when the examiner's finger was removed.

A Dextrostix plastic reagent strip test was carried out, and venous blood was taken for estimation of glucose and calcium concentrations from all infants with tremor, before the next meal. Blood from the reference group infants was taken on the third day before the 10.00 am meal from the same heel puncture that had been used to take blood for thyroid function and phenylketonuria screening tests. Plasma glucose and calcium concentrations were measured by a Technicon SMAC 11.

Hypoglycaemia was defined as a concentration of less than 1·66 mmol/l, and hypocalcaemia as a concentration of less than 1·79 mmol/l.

Informed consent was obtained from all mothers. Statistical analysis was by the unpaired Student's *t* test and 2 × 2 contingency tables.

Table 1 *Characteristics of patients with neonatal tremor and reference group. Figures are given as mean (SD)*

	Birth weight (g)	*Gestational age (weeks)*
Group in which the suckling stimulation test was positive (n=84)	3280 (510)	39·0 (0·9)
Group in which the suckling stimulation test was negative (n=18)	2980 (620)	38·1 (1·1)
Reference group (n=25)	3120 (320)	38·3 (0·7)

There were no significant differences between the neonatal tremor group and reference group.

Results

Two distinct groups of infants were identified from the responses to the suckling stimulation test (table 2). The first comprised 84 infants (82%) in whom the test was positive. They were all normocalcaemic and all but one had normal blood glucose concentrations. The infant whose blood glucose concentration was 1·5 mmol/l was asymptomatic except for the tremor, and the mild hypoglycaemia was corrected by oral feeding. Although the values were within normal limits, plasma glucose concentrations were significantly lower in this group of infants than in the reference group (p<0·006), but there was no difference between the two groups in plasma calcium concentrations.

The second group comprised 18 infants (18%) in whom suckling failed to interrupt the tremor. Thirteen of these had mean (SD) plasma calcium concentrations of 1·44 (0·18) mmol/l (range 1·12–1·75). The other five had a mean glucose concentration of 0·71 (0·29) mmol/l (range 0·5–1·2). None had both abnormalities. The tremors resolved completely after correction of the hypoglycaemia in five infants and hypocalcaemia in 10 infants. In three of the hypocalcaemic infants the tremor continued to occur occasionally, but responded to the suckling stimulation test,

The sensitivity of the suckling stimulation test was 95% with a specificity of 100%.

Discussion

The suckling stimulation test enabled us on the one hand to offer prompt treatment to the infants whose tremor was of true pathological origin, and on the other hand set our minds at rest when we were dealing with physiological tremors. Significantly low concentrations of calcium or glucose as found in 18 infants were detected with a high sensitivity (95%). Only one infant with a positive test was hypoglycaemic.

Table 2 *Calcium and glucose concentrations in the three groups. Figures are given as mean (SD) and range*

	Calcium (mmol/l)	*Glucose (mmol/l)*
Group in which the suckling stimulation test was positive (n=84)	2·11 (0·15), 1·79–2·67	3·16 (0·72),* 1·5–5·2
Group in which the suckling stimulation test was negative:		
Hypocalcaemic subgroup (n=13)	1·44 (0·18),† 1·12–1·75	3·65 (0·37), 2·3–4·1
Hypoglycaemic subgroup (n=5)	2·18 (0·19), 1·90–2·32	0·74 (0·29),† 0·5–1·2
Reference group (n=25)	2·28 (0·12),† 1·89–2·54	3·81 (0·9),*‡ 2·4–5·1

*p<0·006; †‡p<0·001.

The response of the infants with positive reactions to the suckling stimulation test were different. Although within 'normal' limits,[1] their mean glucose concentration was significantly lower than that of the reference group (3·16 (0·7) and 3·84 (0·9) mmol/l, respectively; p<0·006).

Clinical symptoms and signs of neonatal hypoglycaemia may be divided into two main categories; the first comprises those resulting from lack of glucose for central nervous system metabolism such as irritability, bizzare behaviour, convulsions, and coma. The second comprises signs resulting from increased adrenaline secretion such as tremor, jitteriness, pallor, sweating, and tachycardia. Though symptoms of excess adrenaline secretion usually occur with transient mild hypoglycaemia, symptoms arising from central nervous system depression tend to predominate as the severity or duration of the hypoglycaemia increases.[2]

Our results may indicate that the infants responding to the suckling stimulation test had excess adrenaline secretion, which by some unknown mechanism was corrected by suckling. A possible explanation is that the act of suckling raises the concentration of some gastrointestinal substance (a neurotransmitter such as the 14 amino acid gastrin) acting as an adrenaline antagonist.[3] Another explanation is that brain gut peptides may have a direct effect on the neurones in the extrapyramidal system.[4–6] Tremor caused by hypocalcaemia that did not respond to the suckling stimulation test results from a direct effect of low calcium concentration on neuromuscular transmission.

In conclusion we suggest that the suckling stimulation test should be applied to any newborn presenting with neonatal tremor of unknown origin. A positive response to the test combined with a normal plasma glucose concentration measured by Dextrostix plastic reagent strip testing would define the physiological tremor and spare the infant from further investigation.

References

[1] Cornblath M, Pildes R, Schwartz R. Hypoglycemia in infancy and childhood. *J Pediatr* 1973;**83**:692.

[2] LaFranchi S. Hypoglycemia of infancy and childhood. *Pediatr Clin North Am* 1987;**34**:961–81.

[3] Dockray GJ, Vaillant C, Hopkins CR. Biosynthetic relationships of big and little gastrins. *Nature* 1978;**273**:770–2.
[4] Dockray GJ. Brain gut peptides. *Viewpoints on digestive diseases.* 1981;**13**:5–8.
[5] Kreiger DT, Martin JB. Brain peptides. First of two parts. *N Engl J Med* 1981;**304**:876–85.
[6] Kreiger DT, Martin JB. Brain peptides. Second of two parts. *N Engl J Med* 1981;**304**:944–51.

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Effects of ethamsylate on cerebral blood flow velocity in premature babies

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SUMMARY Cerebral blood flow velocity and cardiac output were measured with ultrasound before and 30 minutes after the administration of ethamsylate in a double blind placebo controlled study of 19 very low birthweight infants. No differences were found before or after treatment in either group.

Ethamsylate (diethylammonium 2,5-dihydroxybenzenesulphonate) is a non-steroidal drug that reduces bleeding from small vessels. The results of a double blind multicentre study showed that there were fewer severe periventricular haemorrhages among the survivors of a group of very low birthweight infants treated with this drug shortly after birth.[1] The mode of action remains to be clearly elucidated but experimental evidence shows an inhibition of the products of the prostaglandin pathway.[2] A group of very low birthweight infants with respiratory distress syndrome treated with ethamsylate had lower serum concentrations of immunoreactive prostacyclin metabolite than an untreated control group,[3] and one interpretation was that the drug limited prostacyclin release from ischaemic brain, perhaps preventing bleeding secondary to the presence of this potent vasodilating and platelet disaggregating substance.

Prostaglandins are known to play a part in cerebral blood flow. Indomethacin inhibits prostaglandin production although at a different enzymatic point than that thought to be affected by ethamsylate. Studies in animals, adult humans, and babies attest to the reduction in cerebral blood flow velocity after treatment with indomethacin.[4] Although inhibition of the prostaglandins produced by metabolism of arachidonic acid are not as complete after treatment with ethamsylate as after treatment with indomethacin, it seems plausible that this drug may also reduce cerebral blood flow velocity in the newborn. Periventricular haemorrhages develop early in postnatal life in most infants, therefore a common approach to prevention entails prophylactic administration of the drug to a cohort of babies defined as 'high risk'. Sixty per cent of very low birthweight infants will be given treatment for a condition that they will never develop if this approach is adopted; proposed agents therefore need to be fully investigated.

Patients and methods

Nineteen very low birthweight infants admitted to the Cambridge neonatal intensive care unit who did not have periventricular haemorrhages were studied at a postnatal age of less than 12 hours. Mean (SD) birth weight was 1105 (265) and mean gestational age 28 weeks (range 26–31). Approval for the investigation was granted by the ethical committee of Addenbrooke's Hospital. Measurements of cerebral blood flow velocity and cardiac output were made before and 30 minutes after administration of a solution containing either ethamsylate or saline. Infants were treated with 0·1 ml/kg of solution that contained either 12·5 mg/kg of ethamsylate, or saline alone. The solution was prepared in identical vials by the manufacturers who held the identification code until the end of the study.

Measurement of cerebral blood flow velocity and cardiac output were made by duplex Doppler ultrasound (ATL Mk 600). Doppler signals from the anterior cerebral artery were identified using the real time image and the Fourier transform of the frequency shift was sampled using an Apple IIe microcomputer. This was used to estimate time averaged mean velocity from several consecutive cardiac cycles and also used to calculate the Pourcelot resistance index.[5] This was expressed as the peak systolic velocity minus the diastolic velocity divided by the peak systolic velocity. The same computation, including an angle correction where necessary, was performed for an ascending aortic Doppler sample. This velocity figure, together with an estimate of the aortic size from the internal edge diameter measured by M mode echocardiography, was used to calculate the cardiac output.

Results were analysed by non-parametric statistical tests using the Statistical Package for the Social Sciences. The sign test and the Wilcoxon rank sum test were used to compare pairs of results, and unpaired results were compared by the Mann-Whitney U test.

Results

There was no difference in cardiac output, velocity of blood flow in the cerebral arteries, or the Pourcelot resistance index between the two sets of observations in either the treated or placebo group (table). By chance the ethamsylate treated group had a slightly lower median velocity than the control babies and the velocity in the treated group is significantly lower than in the control group at 30 minutes. This is unlikely to reflect an effect of the drug because the Wilcoxon and sign tests of the paired results are not significant.

The tendency to lower cerebral blood flow velocity in this group was not explained and was not followed by more intraventricular haemorrhages (one in each group).

Discussion

In this group of premature babies ethamsylate had no effect on cerebral blood flow velocity, Pourcelot index, or cardiac output.

Reduction of cerebral blood flow after treatment with indomethacin occurs after a few minutes, and one interpretation is that these effects cannot therefore be due to prostaglandin inhibition, which takes at least 30 minutes and perhaps as long as two hours to occur.[6] An effect mediated by some factor associated with the structure of indomethacin would also explain the observations that a reduction in cerebral blood flow velocity has not been seen with other prostaglandin synthetase inhibitors such as aspirin, and that one hour after the administration of identical doses of indomethacin given at different rates only those infants who received a rapidly administered dose had reduced cerebral blood flow velocity. Aspirin is a more potent inhibitor of prostaglandin synthesis than ethamsylate, and prolongs the bleeding time in the same way as indomethacin, an effect not seen in preterm babies treated with ethamsylate.[3]

The concern about possible ischaemic damage caused by a reduction in cerebral blood flow after rapid administration of indomethacin does not seem to apply to ethamsylate. It may be that a reduction in the incidence of neurodevelopmental handicap will be associated with fewer grade 2 and 3 periventricular haemorrhages in the survivors treated with ethamsylate, but meanwhile it would seem a safe choice for those who wish to use pharmacological prophylaxis in the nursery.

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References

[1] Benson JWT, Drayton MR, Hayward C, *et al.* Multicentre trial of ethamsylate for prevention of periventricular haemorrhage in very low birthweight infants. *Lancet* 1986;ii:1297–1300.

[2] Kovacs L, Falkay G. Ethamsylate as inhibitor of prostacyclin synthesis in pregnant human myometrium. *Experientia* 1981;**37**:1182–3.

[3] Rennie JM, Doyle J, Cooke RWI. Ethamsylate reduces immunoreactive prostacyclin metabolite in low birthweight infants with RDS: possible mode of prevention of intraventricular haemorrhage. *Early Hum Dev* 1986;**14**:239–44.

[4] Evans DH, Levene MI, Archer LNJ. The effect of indomethacin on cerebral blood flow velocity in premature infants. *Dev Med Child Neurol* 1987;**29**:776–82.

[5] Drayton MR, Skidmore R. Vasoactivity of the major intracranial arteries in newborn infants. *Arch Dis Child* 1986;**62**:230–46.

[6] Rennie JM, Doyle J, Cooke RWI. Early administration of indomethacin to preterm infants. *Arch Dis Child* 1986;**61**:233–8.

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Table *Cerebral blood flow velocity. Pourcelot index, and cardiac output in the two groups. Values are given as median (interquartile range)*

	Group given ethamsylate		*Group given placebo*	
	Before treatment	*After treatment*	*Before treatment*	*After treatment*
Cerebral blood flow velocity (cm/s)	2·83 (2·34–3·5)	2·18 (1·95–3·15)*	3·55 (2·6–5·75)	4·3 (3·4–5·2)*
Pourcelot index	0·93 (0·77–0·98)	0·88 (0·81–1·0)	0·92 (0·7–0·98)	0·79 (0·79–0·9)
Cardiac output (ml/kg/min)	201 (146–220)	144 (130–193)	158 (133–253)	138 (127–227)

*$p<0·05$, Mann-Whitney U test.

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Simple test to distinguish between surgical and non-surgical pneumoperitoneum in ventilated neonates

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SUMMARY A simple and accurate method is described for rapid differentiation of surgical and nonsurgical pneumoperitoneum by measuring the partial pressure of oxygen in intraperitoneal air with a blood gas analyser. Results in five ventilated neonates in whom this distinction was not possible by clinical or radiographic means are presented.

Clinical signs and symptoms with or without radiographic findings usually allow differentiation of pneumoperitoneum resulting from air dissecting transdiaphragmatically from the so called surgical pneumoperitoneum caused by leakage of gas from perforated bowel.[1-3] In some ventilated infants a clear distinction cannot be made between the two types of pneumoperitoneum using these factors alone, and Chang and Hernandez[4] suggested a rapid method of differentiation by measuring the oxygen concentration in the intraperitoneal air. We propose a less cumbersome and probably more reliable method.

Patients and methods

Between January 1985 and March 1988 nine out of 580 ventilated neonates in our neonatal intensive care unit developed radiographic evidence of pneumoperitoneum (table 1). In seven of these (cases 1–7) the pneumoperitoneum was caused by intestinal perforation, whereas in the other two the nonsurgical pneumoperitoneum was the result of dissecting extra-alveolar air.

In four of the seven babies with gastrointestinal pneumoperitoneum (cases 1–4) no alveolar air leak syndrome was present and sufficient clinical or radiographic findings were available for a certain preoperative diagnosis. In the remaining three (cases 5–7) chest radiographs confirmed the presence of pulmonary air leak syndrome (pneumothorax, pneumomediastinum, or interstitial emphysema, or a combination) in association with hyaline membrane disease before the finding of intraperitoneal gas. In these three neonates, as well as in the two babies with pneumoperitoneum of respiratory origin (cases 8 and 9) neither clinical, nor radiographic differentiating features were available

Table 1 *Clinical and radiographic findings in nine ventilated newborn infants with pneumoperitoneum*

Case No	*Extra alveolar air*	*Differentiating features*	*Postoperative diagnosis*
1	None	Pneumatosis intestinalis	Necrotising enterocolitis
2	None	Absence of gastric air/fluid level	Gastric perforation
3	None	Intraperitoneal air/fluid level	Colon perforation
4	None	Perforation of appendix identified by contrast examination	Neonatal appendicitis
5	Bilateral pneumothorax and pneumomediastinum	None	Solitary ileal perforation
6	Unilateral pneumothorax, pneumomediastinum, and pulmonary interstitial emphysema	None	Solitary ileal perforation
7	Pneumomediastinum and pulmonary interstitial emphysema	None	Solitary ileal perforation
8	Bilateral pneumothorax,, pneumomediastinum, and pulmonary interstitial emphysema	None	No operation
9	Bilateral pneumothorax, pneumomediastinum, and pulmonary interstitial emphysema	None	No operation

that permitted specific identification of the origin of the intraperitoneal gas.

The partial pressure of oxygen in the inspired air (PIO_2) may be calculated when the oxygen concentration in the inspired air (FiO_2) and barometric pressure (PB) are known: $PIO_2=FiO_2\times(PB-6{\cdot}25)$.[5] According to the physical laws of Dalton and Henry, partial pressure of oxygen in alveolar gas (PAO_2) must be less than that of the inspired gas. It may be calculated from the *alveolar air equation*: $PAO_2=PIO_2-PACO_2/R$ (R=respiratory gas exchange ratio$\approx$0·8).[5]

In cases 5 to 9, a 20 gauge Quickcath was inserted into the peritoneal cavity at the right lower quadrant after emptying the urinary bladder. The catheter was attached to a three way stopcock and a 10 ml syringe. An aliquot of intraperitoneal air was aspirated and the stopcock closed before removal of the catheter. Aspirated air was gently introduced into the syringe port of the pH blood gas analyser (Corning 178, Corning Medical and Scientific) and partial pressure of intraperitoneal oxygen was measured and recorded without removing the syringe until the readout had stabilised (readouts endpoint).

Results

Table 2 shows the results of the calculated measurements corresponding to the oxygen concentration delivered by the respirator, and the results of the partial pressure of intraperitoneal oxygen measured by the blood gas analyser. In the three infants with surgical pneumoperitoneum (cases 5–7) the partial pressure of oxygen delivered by the ventilator (and the partial pressure of oxygen in the alveolar gas) far exceeded the value of the intraperitoneal oxygen (swallowed room air). In contrast, in the two infants (cases 8 and 9) with ventilator induced pneumoperitoneum, the intraperitoneal oxygen tension far exceeded the maximal pressure of oxygen in room air (19·85 kPa) and reflected clearly the calculated partial pressure of oxygen in alveolar gas deduced from the oxygen concentration delivered by the respirator.

Discussion

Diagnostic errors in differentiating pneumoperitoneum of intestinal from that of respiratory origin in ventilated neonates have been well documented.[1–4 6] Because clinical and radiographic criteria are not always adequate, some infants have been needlessly submitted to surgical intervention, and some others to an unacceptable delay in diagnosis, allowing general peritonitis to develop.

In the event that a clear distinction cannot be made, abdominal paracentesis should be performed in order to measure oxygen concentration in the intraperitoneal gas as suggested by Chang and Hernandez.[4] We have suggested a less cumbersome method based on measurement of the partial pressure of intraperitoneal oxygen with a blood gas analyser.

References

[1] Leonidas JC, Hall RT, Rhodes PG, Amoury RA. Pneumoperitoneum in ventilated newborns. *Am J Dis Child* 1974;**128:** 677–80.

[2] Kaufman RA, Kuhns LR, Poznanski AK. Gastrointestinal perforation without intraperitoneal air-fluid level in neonatal pneumoperitoneum. *AJR* 1976;**127:**915–21.

[3] Ensing G, Koppe JG, van Putten WJ. Pneumoperitoneum in the newborn. *J Pediatr Surg* 1974;**9:**547–50.

[4] Chang JHT, Hernandez J. Ventilator-induced pneumoperitoneum—a rapid diagnosis. *Pediatrics* 1980;**66:**135–6.

[5] Burgess WR, Chernick V. Pulmonary physiology and the child. In: Burgess WR, Chernick V, eds. *Respiratory therapy in newborn infants and children*. 2nd ed. Stuttgart: Georg Thieme, 1986:12–8.

[6] Knight PJ, Abdenour G. Pneumoperitoneum in the ventilated neonate: respiratory or gastrointestinal origin? *J Pediatr* 1981; **98:**972–4.

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Table 2 *Calculated partial pressure of oxygen in inspired air, partial pressure of oxygen in alveolar gas, and partial pressure of intraperitoneal oxygen measured by blood gas analyser*

Case No	*Oxygen concentration in inspired air*	*Partial pressure of oxygen in inspired air (kPa)*	*Partial pressure of oxygen in alveolar gas (kPa)*	*Partial pressure of intraperitoneal oxygen (kPa)*
5	0·85	80·8	74·1	7·73
6	0·35	33·3	26·7	6·53
7	0·65	61·75	55·0	5·60
8	0·60	56·9	50·3	43·33
9	0·80	76·0	69·3	66·05

Assuming mean barometric pressure of 101·3 kPa, $PACO_2$ of 5·33 kPa, and a respiratory gas exchange ratio of 0·8 ($PAO_2=FiO_2\times(101{\cdot}3-6{\cdot}25)-5{\cdot}33/0{\cdot}8$). 1 mm Hg=0·1333 kPa.

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Neonatal bilateral diaphragmatic paralysis caused by brain stem haemorrhage

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SUMMARY We describe a neonate with severe bilateral diaphragmatic paralysis caused by haemorrhage in the lower brain stem. To our knowledge this association has not been previously reported in the English medical literature.

Bilateral diaphragmatic paralysis in the neonate is a life threatening situation.[1 2] It is usually a result of birth trauma to the cervical nerve roots or cervical spine, although agenesis of the phrenic nerve has also been reported,[1] and in some cases no cause could be determined.[2 3]

Case report

A boy, weighing 3600 g, was delivered at 42 weeks' gestation after an uncomplicated pregnancy. After delivery intubation was required because of delayed onset breathing, and the endotracheal tube was suctioned because of the presence of heavy meconium stained amniotic fluid. The Apgar scores were 4 and 8 at 1 and 5 minutes, respectively. Physical examination after delivery showed mild respiratory distress, and there was mild hypotonia of both legs. No signs of brachial plexus damage were noted.

At 1 hour of life respiratory distress increased and paradoxical respiratory movements were noted. Values for blood gases were within the normal range. A chest radiograph showed elevation of both diaphragms to the level of the fifth rib, without signs of meconium aspiration (fig 1). Chest fluoroscopy showed no spontaneous diaphragmatic movements, but there was paradoxical upward movement of both diaphragms during inspiration. Respiratory difficulty increased, and mechanical ventilation had to be instituted.

At 14 hours of life the sucking reflex disappeared. Ultrasonography showed no intracranial haemorrhage; the spinal fluid was normal. Computed tomography of the head, performed on the 10th day of life (fig 2) showed a large asymmetric area of increased density in the medulla oblongata extending more to the right, a finding that was interpreted

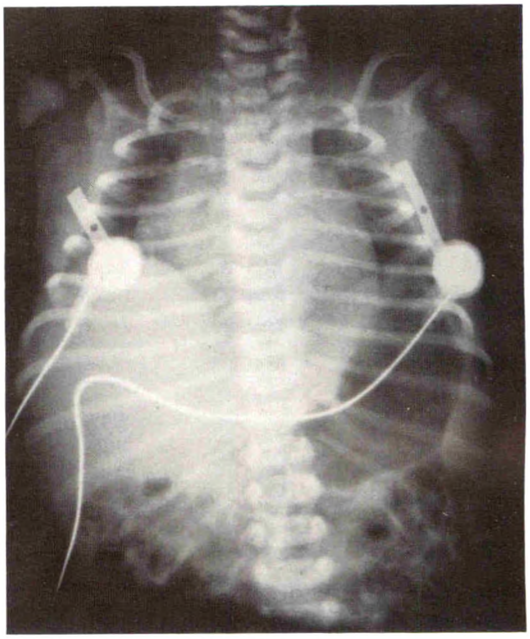

Fig 1 *Radiograph of chest on the 1st day of life showing the high position of both diaphragms.*

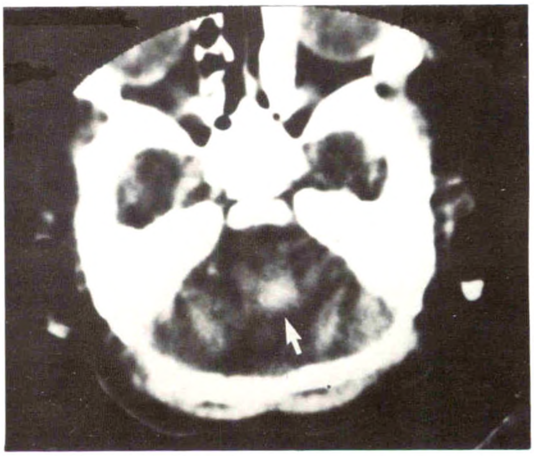

Fig 2 *First computed tomogram showing hyperdense region within the right medulla oblongata (arrow).*

as haemorrhage. The pons, fourth ventricle, and basal cisterns were normal.

On the 3rd week of life, generalised hypotonia was observed and there were fasciculations of the tongue. Electromyographic studies showed normal conduction velocities in the extremities but there were signs of denervation (fibrillation potentials) in the sternocleidomastoid and trapezius muscles. Attempts to evoke phrenic nerve activity failed. Muscle enzymes values were within normal limits. Histological and electron microscopical studies of muscle gave normal results.

At the age of 6 weeks, the fasciculations of the tongue subsided. A repeat electromyographic study showed diaphragmatic activity after stimulation of the phrenic nerve, but there were still no spontaneous diaphragmatic movements. A computed tomogram of the head showed resorption of the haemorrhagic focus in the medulla, with a reduction in brain stem size and widening of prepontine and quadrigeminal cisterns. Higher sections showed diffuse atrophic changes. At the age of 7 months the computed tomogram showed more pronounced cerebral atrophic changes.

At 3 months of life the baby could be weaned from the respirator, although moderate respiratory difficulty persisted over the next several months. Sucking and swallowing ability improved steadily.

Partial diaphragmatic paralysis persisted until the age of 11 months, when breathing became practically normal, with return of the diaphragms to a normal position on chest radiography and fluoroscopy. During the above period, the infant received intensive respiratory physiotherapy and stimulation. His mental development progressed satisfactorily, but gross motor achievements remained slightly delayed.

Discussion

The diaphragm has an essential role as a respiratory muscle in neonates. The major changes in thoracic volume in neonates are the result of diaphragmatic contraction.[1] Diaphragmatic malfunction in neonates, by either a primary diaphragmatic muscular problem or secondary to phrenic nerve paralysis (unilateral or bilateral), may produce severe cardiopulmonary embarrassment and may result in death if early supportive treatment is not carried out.[1] Brain stem haemorrhage has not previously been reported as a cause of paralysis of the diaphragm in the neonate.

Posterior fossa haemorrhages are rare in the newborn. In one series 17 neonates out of 700 were found to have a posterior fossa haemorrhage on computed tomography (six of these infants were premature).[4] These posterior fossa haemorrhages were either in the subdural space or intracerebellar. Brain stem haemorrhage was not present in any of them. The prognosis in these neonates was poor: seven died, and only three of the survivors were normal at follow up. In another series, in a postmortem study of 105 neonates and fetuses, intracerebellar haemorrhage was found in two cases; the pathogenesis of the posterior fossa haemorrhage was attributed to birth trauma or prematurity.[5] The aetiology of posterior fossa haemorrhage in the premature may be similar to that of the subependymal haemorrhage in the cerebral hemispheres.[4] Perinatal asphyxia and hypoxic-ischaemic brain damage may be an important cause of posterior fossa haemorrhage,[6] and may also explain the development of cerebral atrophy in our case. In the English medical literature we could not find another case in which the diagnosis of brain stem haemorrhage was made during life.

In the present case, the baby suffered from perinatal distress with respiratory difficulty immediately after birth, and bilateral diaphragmatic paralysis was diagnosed. Cervical trauma causing phrenic nerve injury could be excluded; peripheral phrenic injury was unlikely, as cervical phrenic nerve stimulation resulted in diaphragmatic contractions. The computed tomogram disclosed a large haemorrhage within the medulla oblongata; an insult to this strategic neurological site may cause severe functional or structural damage endangering the newborn's life. The motor cranial nuclei IX, X, XI, and XII in the medulla oblongata programme and activate the phrenic and intercostal nerves. The motor neurons to the phrenic nerve (in C3, C4, and C5), to the intercostal muscles and presumably, to the accessory muscles of respiration are all under the control of respiratory centres in the medulla oblongata. These may have become damaged by the haemorrhagic lesion in our patient.

This infant displayed respiratory distress due to neurogenic diaphragmatic paralysis, which improved and returned to normal over the following months, after resorption of a medullary haemorrhage. The temporary clinical appearance of fasciculations of the tongue and electrophysiological fibrillations in the muscles innervated by the accessory nerve are fully consistent with the localisation of the haemorrhage in the medulla, with functional injury of the IX, X, and XII motor nuclei.

The commonly recognised causes of diaphragmatic paralysis are local pathology of the diaphragm (muscular hypoplasia) or phrenic nerve pathology (injury or agenesis). This report describes a higher level, intracranial pathology, as a cause of peripheral functional diaphragmatic paralysis. The

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Current topic

Fetal growth signals

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Human fetal growth is not uniform. Tissue patterns and organ primordia are established during embryogenesis, then from the end of the first trimester and throughout the second the fetus undergoes massive hyperplasia. In the third trimester further organ modelling and functional maturation occur in preparation for extrauterine life. Each aspect of development requires orchestrated intercellular signalling at two levels. The release of peptide growth factors and the modulation of an extracellular matrix are paracrine actions that occur within cell populations and between adjacent germ layers. In contrast, endocrine hormones may stimulate growth non-specifically or promote specific maturational events. The interactions between paracrinology, endocrinology, and environmental constraints to growth during normal and abnormal fetal development have been reviewed in detail recently.[1 2] In this commentary emphasis has been placed on new concepts of embryonic and fetal growth control.

Paracrinology

Peptide growth factors act on cell cultures in vitro to stimulate differentiation, functional activity, and chemotaxis as well as causing cell proliferation.[3] Though this has led to speculation that peptide growth factors are fundamental to prenatal development, the technology and purified peptides to test the relevant hypotheses in vivo have only recently become available.

FIBROBLAST GROWTH FACTOR AND TRANSFORMING GROWTH FACTOR β

It is now clear that at least two peptide growth factors, fibroblast growth factor and transforming growth factor β are present early in embryogenesis and are potentially concerned with germ layer separation. Fibroblast growth factor has been identified in chick embryo from day 11 of incubation and messenger ribonucleic acid (mRNA) for fibroblast growth factor has been located in the Xenopus blastula.[4] Messenger RNA encoding a transforming growth factor β like peptide is found in the vegetal pole ectoderm of the early Xenopus embryo and is transcribed by a maternally derived gene designated Vgl. An ectodermal cell line, XTC, from the metamorphosing tadpole releases a transforming growth factor β like peptide in vitro.[5] In the 11–18 day fetal mouse transforming growth factor β can be detected in bone and connective tissue, particularly that derived from neural crest such as palate, larynx, facial mesenchyme, and teeth.[6] Staining was most intense at sites of tissue morphogenesis affecting mesodermal and epithelial interaction such as hair follicles, teeth, and secondary palate.

Recently both fibroblast growth factor and transforming growth factor β have been shown to exert remarkable effects on embryonal morphology. Induction of mesoderm in the amphibian embryo depends on morphogens from the ectoderm of the vegetal pole diffusing to cause mesodermal development in the ectoderm of the animal pole. Fibroblast growth factor induces mesoderm in Xenopus animal pole ectoderm in vitro, the structures developing being mainly mesenchyme, mesothelium, and blood cells enveloped in ectoderm.[7] Transforming growth factor β also induces mesoderm in Xenopus ectoderm, especially muscle, which was identified by the presence of α actin mRNA.[5] The most potent natural source of mesoderm inducing activity is conditioned medium from Xenopus XTC cell cultures, and this can be blocked by transforming growth factor β antiserum. Fibroblast growth factor promotes mitosis, cell migration, invasion, and production of plasminogen activator by vascular endothelial cells, which are all necessary features of angiogenesis in vivo.[8] It also induces the differentiation of chondrocytes, preadipocytes, astrocytes, and oligodendrocytes. Not all actions of fibroblast growth factor on tissue differentiation are augmentative, as it attenuates the differentiation and fusion of fetal myoblasts and decreases concentrations of muscle specific enzymes such as myokinase. Of particular relevance to embryogenesis is the avid association of fibroblast growth factor with heparin sulphate, a glycosaminoglycan produced in large amounts by vascular and corneal endothelium and a structural component of their extracellular matrix.

Fibroblast growth factor may act in a novel way because its gene does not encode the conventional peptide sequence that is necessary for vesicle associated exocytosis.[8] The peptide may leave the cell together with matrix molecules, and either react immediately with fibroblast growth factor receptors as an autocrine stimulus, or remain stored within the matrix. Since organogenesis entails cells interacting with newly formed matrix to promote stem cell growth and stabilise phenotype, it is possible that fibroblast growth factor might influence differentiation from a matrix store.

INSULIN LIKE GROWTH FACTORS

There seems to be no report of insulin like growth factor being present in embryos before separation of the germ layers. Studies with embryonal mouse carcinoma cell lines suggest that insulin like growth factor II occurs only after differentiation of the pluripotential ectodermal cells by exposure to retinoic acid to yield cells with mesodermal or endodermal phenotypes. Insulin like growth factor and epidermal growth factor may have a fundamentally different role from transforming growth factor β and fibroblast growth factor. They are expressed during and after the condensation of tissues and may influence expansion and differentiation of stem cell populations.

In the human fetus in the early second trimester, insulin like growth factors have been localised by immunohistochemistry to epithelia within the gut, kidney, and lung, and to hepatocytes, fetal zone adrenal cells, skeletal, and cardiac muscle fibres, haemopoietic cells, and dermis.[9] This distribution may be partly due to a complex of insulin like growth factor with specific binding protein attaching avidly to certain cell membranes. The binding protein has been shown to potentiate the biological actions of insulin like growth factors in vitro.[10] The tissue distribution of insulin like growth factor binding protein in the human fetus mirrors that of insulin like growth factor itself suggesting that the two coexist as a complex in or on certain cells.[11] This may reflect sequestration of insulin like growth factor, as in situ hybridisation studies show most insulin like growth factor mRNA to be in fibrous mesenchyme adjacent to the cell types positive for insulin like growth factor.[12]

Most insulin like growth factor mRNA in rat or human fetuses encodes insulin like growth factor II, but insulin like growth factor I mRNA is detectable in the rat embryo from day 11 and in the human fetus from late in the first trimester. In both chick and human development insulin like growth factors are strongly associated with populations of differentiated functional cells, suggesting that their role is to support this phenotype. Both insulin like growth factors are not only mitogenic but also induce differentiation.[2] Insulin like growth factor I induces the fusion of myoblasts and the appearance of muscle specific enzymes in postmitotic contractile myotubes, and is 100 times more potent than insulin like growth factor II in this respect. Insulin like growth factor I augments the action of follicle stimulating hormone during differentiation of ovarian granulosa cells, leading to an increase in luteinising hormone receptors and sex steroid accumulation. Whereas insulin like growth factor I promotes the differentiation of fetal brain astrocytes into oligodendrocytes, insulin like growth factor II synergises with nerve growth factor to promote neurite outgrowth from sensory and sympathetic ganglia. Both insulin like growth factor I and II increase extracellular matrix synthesis by connective tissues, particularly chondrocytes.

Plasma insulin like growth factor I and II in the human fetus rise gradually until full term, when insulin like growth factor I, but not insulin like growth factor II correlates with birth weight. If we accept that the main action of insulin like growth factors is a local one, the biological interpretation of circulating concentrations is difficult. Nevertheless there is a sharp rise in plasma insulin like growth factor II in the fetal rat, lamb, and guinea pig that is coincident with the onset of gluconeogenesis.

EPIDERMAL GROWTH FACTOR AND TRANSFORMING GROWTH FACTOR α

Epidermal growth factor is also associated with cell differentiation and maturation during embryogenesis. In the mouse embryo the first differentiated cell type, primary trophoectoderm, possesses epidermal growth factor receptors that may be activated by either epidermal growth factor or its analogue transforming growth factor α. Although transforming growth factor α concentration is highest in the mouse embryo at day 7, transforming growth factor α mRNA cannot be detected at this time but is abundant in the maternal decidua, especially adjacent to the embryo.[13] Decidual expression began after implantation, peaked at day 8, then slowly declined with decidual reabsorption. Transforming growth factor α may therefore be available to the embryo from a maternal source during gastrulation and neurulation. By early in the second trimester, epidermal growth factor is found in the human fetus in the gastrointestinal tract, kidney, pituitary gland, trachea, and placenta.[14]

In the fetus epidermal growth factor affects the growth, differentiation, and function of epithelial cells.[2] When given parenterally to the fetal lamb, it stimulates skin hypertrophy and growth of the viscera. In both the rabbit and the lamb it causes lung epithelial maturation and surfactant production. In vitro, it is mitogenic and can influence differentiation; it inhibits glucocorticoid induced keratinisation of embryonic chick skin. Receptors for epidermal growth factor are abundant in human placenta, especially on the microvillous plasma membranes facing the maternal circulation and the basolateral membranes facing the fetal circulation. It is not surprising, therefore, that studies with isolated trophoblasts or placental cultures have shown that epidermal growth factor modulates trophoblast differentiation and function. In homogenous cultures of full term trophoblasts it caused a dose related release of human placental lactogen and human chorionic gonadotrophin.[15] Trophoblasts differentiated into a syncytium of cytotrophoblasts but did not multiply. It is not known if the epidermal growth factor acting on placenta in vivo is placental, or comes from elsewhere in the fetus or mother.

Endocrinology

Because endocrinology has been with us for much longer than paracrinology the role of the endocrines in fetal growth control is understood in a more balanced manner and has been the subject of regular detailed review. Conceptual changes concerning endocrine growth control are evolving more slowly than those in paracrinology and arguably the most topical facet of fetal endrocrinology is placental hormone production. Otherwise most of the points made here count as fine tuning of established dogma.

Whereas fetal growth continues in the absence of hormones such as thyroxine, insulin, or the pituitary hormones (none of which cross the placenta in physiologically significant amounts), optimal growth depends on all of these. Insulin is necessary to promote anabolism, thyroid hormones stimulate neuronal maturation, and pituitary hormones are necessary for enzymic and gonadal development.

INSULIN

Insulin is detectable in the human pancreas from eight weeks', and in fetal plasma from 12 weeks' gestation but may well be present in the early embryo because mRNA for proinsulin has been identified in embryonic chick yolk sac. During embryogenesis insulin may be both a mitogen and a metabolic hormone. At physiological concentrations of 1–10 nM insulin stimulated tissue growth, metabolism, and muscle differentiation in four day chick embryos, and stimulated the appearance of choline acetyltransferase, a differentiation marker in retinal neurones, at seven days.[16 17] Insulin stimulated glucose oxidation in chick embryos as early as gastrulation at 18 hours, and neurulation at 24 hours.[18]

The role of insulin as a fetal metabolic hormone varies between species and depends as much on the ontogeny of receptor and post receptor links as on the presence of insulin itself. Despite abundant insulin receptors on full term fetal rat hepatocytes, insulin had little effect on glucose incorporation into glycogen or glycogen synthetase activity. When fetal lambs in the third trimester were placed on a glucose clamp and infused with insulin there was a 13% rise in oxygen consumption, a 106% increase in glucose uptake, and an 83% increase in glucose utilisation.[19] The ability of insulin to promote fetal adiposity is well recognised clinically, and has been confirmed experimentally. Insulin infusion into the monkey fetus caused a fall in plasma free fatty acids and an increase in hepatic lipogenic enzyme activity.

Recent experiments have added to our sparse knowledge of human fetal insulin secretion. Perfusion of isolated islet clusters from fetuses of less than 16 weeks' gestation caused a small monophasic rise in insulin secretion within 30 minutes of a glucose challenge.[20] The response increased at 17 weeks' gestation, but only became biphasic in the perinatal period. Other secretogogues that may be more physiologically relevant to the fetus, such as amino acids, were not tested.

The mitogenic action of insulin in physiological concentrations seen during embryogenesis is retained by only a few tissues (such as liver) in later fetal life. Hypoinsulinaemia is, however, invariably associated with a reduction in circulating insulin like growth factor I irrespective of whether the availability of nutrients is also decreased, as seen after ligation of the uterine vessels in the rat,[21] or if fetal glycaemia is maintained, as in the fetal lamb that has undergone pancreatectomy.[22] Because insulin has no direct action on release of insulin like growth factor from fetal human or rat connective tissues or hepatocytes in vitro, it seems likely that the effect of insulin on insulin like growth factor expression is through stimulation of nutrient uptake and utilisation.

The idea that insulin is permissive for fetal growth is supported by the clinical evidence that fetal hyperinsulinaemia, though it causes adiposity, has little effect on lean body mass.[23] Experiments in which fetal monkeys were made chronically hyperinsulinaemic led to similar conclusions. These observations in no way detract from the impact of insulin on fetal morbidity. Hyperinsulinaemia inhibits development of surfactant, which contributes to the increased incidence of respiratory distress syndrome in infants of diabetic mothers. Fetal polycythaemia can result in neonatal problems caused by hyperviscosity and hyperbilirubinaemia. The polycythaemia may arise from the comparative hypoxia caused by increased glucose utilisation that in turn causes increased erythropoietin synthesis or direct stimulation of erythropoiesis by insulin. Human fetal and neonatal erythroid progenitor cells depend less on erythropoietin for growth in vitro than those from adults[24] and insulin, in the presence of erythropoietin, stimulated late erythroid progenitor cell growth in vitro from fetal blood taken between 21 weeks' gestation and full term.[25]

GROWTH HORMONE

Growth hormone is found in the human fetal pituitary gland and circulation from at least 10 weeks' gestation, and by mid pregnancy plasma growth hormone concentrations are at a peak of more than 200 mU/l. Despite this plethora, pituitary growth hormone seems to have little influence on fetal development, possibly because growth hormone receptors do not appear in connective tissue before birth in the mouse, rat, or sheep and not until at least the second trimester in humans. Growth hormone is without effect on human fetal muscle cell growth in vitro, but does stimulate DNA synthesis together with release of insulin like growth factor I, by isolated human fetal hepatocytes from as early as 12 weeks' gestation.[26 27] Immunological neutralisation of the insulin like growth factor release blocked the mitogenic effect of the growth hormone, showing an indirect mechanism of action. Growth hormone binding sites are found on hepatic cell membranes from the end of the first trimester.[28]

Growth hormone and insulin may cooperate to augment fetal growth indirectly. Growth hormone promotes β cell replication and insulin release in pancreatic islet cultures from human fetuses of 12 to 25 weeks' gestation.[29] The β cell is known to contain and release insulin like growth factor I at this time, but it is not known whether insulin like growth factor I mediates the mitogenic action of growth hormone. If growth hormone interacts with islet cells in utero, the growth hormone molecule concerned might be pituitary or placental in origin.

PLACENTAL HORMONES

The human placenta contains a growth hormone like molecule which differs from pituitary growth hormone at 13 amino acids and is a product of the variant growth hormone gene, human growth hormone V that remains dormant in the pituitary. Placental growth hormone is secreted copiously into the maternal circulation towards full term, but disappears within one hour of delivery.[30] Attempts to measure placental growth hormone in the fetus have been bedevilled by the abundance of pituitary growth hormone, and it is not yet appropriate to speculate on its role in fetal life.

Human placental lactogen has 85% homology in amino acid sequence with growth hormone and is released into both maternal and fetal compartments. A role for human placental lactogen in fetal development seemed unlikely because of its low somatotropic activity in classical growth hormone bioassays, such as tibial growth in the rat after hypophysectomy. Such test systems may be inappropriate because they measure the interaction of human placental lactogen and growth hormone receptors, and take no account of the possibility of specific human placental lactogen receptors in fetal tissues.

Random measurements of fetal serum human placental lactogen in the second trimester are between 50 and 200 ng/ml. Human placental lactogen in this concentration range promotes DNA synthesis and cellular anabolism in isolated human connective tissues and hepatocytes.[26 27] The actions on DNA synthesis were mediated by a paracrine release of insulin like growth factor peptides. Specific binding sites for human placental lactogen are present on cell membranes from second trimester fetal liver and skeletal muscle, and receptor numbers increase with body weight between 12 and 19 weeks' gestation.[28] Although the mechanisms for human placental lactogen to potentiate fetal growth are there, its role in utero remains uncertain because reports exist of healthy term infants born to women with placentas lacking the two human placental lactogen genes. In these circumstances other related peptides, such as placental growth hormone, may play a compensatory part.

Comment

Fetal growth is coordinated by spatial and temporal interactions of cell populations with extracellular matrix components and peptide growth factors. In the early embryo the main prerequisites for success are adequate nutrition and gaseous exchange. By the end of the first trimester a complex signalling network has developed, in which fetal and placental hormones interact with tissue growth factors. Hormones act in two ways: they may stimulate growth diffusely or they may act specifically to induce cellular maturation for extrauterine life. What is less clear is the degree of interdependence of endocrine and paracrine pathways. Postnatally they can be hierarchical, as in the control of insulin like growth factor expression by growth hormone, or synergistic as in the interaction between follicle stimulating hormone and insulin like growth factor I during granulosa cell differentiation.

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References

[1] Milner RDG, Hill DJ. Interaction between endocrine and paracrine peptides in prenatal growth control. *Eur J Pediatr* 1987;**146:**113–22.

[2] Hill DJ, Milner RDG. Mechanisms of fetal growth. In: Brook CGD, ed. *Clinical paediatric endocrinology* 2nd ed. Oxford: Blackwell, 1988:3–31.

[3] James R, Bradshaw RA. Polypeptide growth factors. *Ann Rev Biochem* 1984;**53:**259–92.

[4] Kimelman D, Kirschner M. Synergistic induction of mesoderm by FGF and TGF-Beta and the identification of an mRNA coding for FGF in the early xenopus embryo. *Cell* 1987;**51:** 869–71.

[5] Rosa F, Roberts AB, Danielpour D, Dart LL, Sporn MB, Dawid IB. Mesoderm induction in amphibians: the role of TGF-B2-like molecules. *Science* 1988;**239:**783–5.

[6] Heine UI, Munoz EF, Flanders KC, *et al*. Role of transforming growth factor-β in the development of the mouse embryo. *J Cell Biol* 1987;**105:**2861–76.

[7] Slack JMW, Darlington BG, Heath JK, Godsave SF. Mesoderm induction in early xenopus embryos by heparin-binding growth factors. *Nature* 1987;**326:**197–201.

[8] Gospodarowitz D, Neufeld G, Schweigerer L. Fibroblast growth factor: structural and biological properties. *J Cell Physiol* 1987;Suppl 5:15–26.

[9] Han VKW, Hill DJ, Strain AJ, *et al*. Identification of somatomedin/insulin-like growth factor immunoreactive cells in the human fetus. *Pediatr Res* 1987;**22:**245–9.

[10] Elgin RE, Busby WH, Clemmons DR. An insulin-like growth factor (IGF) binding enhances the biological response to IGF I. *Proc Natl Acad Sci USA* 1987;**84:**3254–8.

[11] Hill DJ, Wilson S, Strain AJ, Clemmons DR. Immunolocalization of insulin-like growth factor binding protein (IGF-BP) in human fetal tissues. *J Endocrinol* 1988;**116:**(suppl):244.

[12] Han VKM, D'Ercole AJ, Lund PK. Cellular localisation of somatomedin (insulin-like growth factor) messenger RNA in the human fetus. *Science* 1987;**236:**193–7.

[13] Han VKM, Hunter ES, Pratt RM, Zendegui JG, Lee DC. Expression of rat transforming growth factor alpha mRNA during development occurs predominantly in the maternal decidua. *Mol Cell Biol* 1987;**7:**2335–43.

[14] Kasselberg AG, Orth DN, Gray ME, Stahlman MT. Immunocytochemical localization of human epidermal growth factor/urogastrone in several human tissues. *J Histochem Cytochem* 1985;**33:**315–22.

[15] Morrish DW, Bhardwaj D, Dabbagh LK, Marusyk H, Siy O. Epidermal growth factor induces differentiation and secretion of human chorionic gonadotropin and placental lactogen in normal human placenta. *J Clin Endocrinol Metab* 1987;**65:**1282–90.

[16] Girbau M, Gomez JA, Lesniak A, De Pablo F. Insulin and insulin-like growth factor I both stimulate metabolism, growth, and differentiation in the postneurula chick embryo. *Endocrinology* 1987;**121:**1477–82.

[17] Kyriakis JM, Hausman RE, Peterson SW. Insulin stimulates choline acetyltransferase activity in cultured chicken retina neurons. *Proc Natl Acad Sci USA* 1987;**84:**7463–7.

[18] Baroffio A, Raddatz E, Markert M, Kucera P. Transient stimulation of glucose metabolism by insulin in the 1-day chick embryo. *J Cell Physiol* 1986;**127:**288–92.

[19] Hay WW, Meznarich HK. The effect of hyperinsulinaemia on glucose utilization and oxidation and on oxygen consumption in the fetal lamb. *Q J Exp Physiol* 1986;**71:**689–98.

[20] Otonkoski T, Andersson S, Knip M, Simell O. Maturation of insulin response to glucose during human fetal and neonatal development. Studies with perifusion of pancreatic islet like cell clusters. *Diabetes* 1988;**37:**286–91.

[21] Vileises RA, D'Ercole AJ. Tissue and serum concentrations of somatomedin-C/insulin-like growth factor I in fetal rats made growth retarded by uterine vessel ligation. *Pediatr Res* 1986;**20:** 126–30.

[22] Gluckman PD, Butler JH, Comline R, Fowden A. The effects of pancreatectomy on the plasma concentrations of insulin-like growth factors 1 and 2 in the sheep fetus. *J Dev Physiol* 1987;**9:**79–88.

[23] Naeye RC. Infants of diabetic mothers: a quantitative morphological study. *Pediatrics* 1965;**35:**980–8.

[24] Linch DC, Knott LJ, Rodeck CH, Heins ER. Studies of circulating hemopoietic progenitor cells in human fetal blood. *Blood* 1982;**59:**976–9.

[25] Perrine SP, Greene MF, Lee PDK, Cohen RA, Faller DV. Insulin stimulates cord blood erythroid progenitor growth: evidence for an aetiological role in neonatal polycythaemia. *Br J Haematol* 1986;**64:**503–11.

[26] Hill DJ, Crace CJ, Milner RDG. Incorporation of [3H] thymidine by isolated fetal myoblasts and fibroblasts in response to human placental lactogen (HPL): possible mediation of HPL action by release of immunoreactive SM-C. *J Cell Physiol* 1985;**125:**337–44.

[27] Strain AJ, Hill DJ, Swenne I, Milner RDG. The regulation of DNA synthesis in human fetal hepatocytes by placental lactogen, growth hormone and insulin-like growth factor I/somatomedin-C. *J Cell Physiol* 1987;**132:**23–40.

[28] Hill DJ, Freemark M, Strain AJ, Handwerger S, Milner RDG. Placental lactogen and growth hormone receptors in human fetal tissues: relationship to fetal plasma human placental lactogen concentrations and fetal growth. *J Clin Endocrinol Metab* 1988;**66:**1283–90.

[29] Sandler S, Andersson A, Korsgren O, Tollemar J, Petersson B, Groth C-G, Hellerstrom C. Tissue culture of human fetal pancreas: growth hormone stimulates the formation and insulin production of islet-like cell clusters. *J Clin Endocrinol Metab* 1987;**65:**1154–8.

[30] Frankenne F, Closset J, Gomez F, Scippo ML, Smal J, Hennen G. The physiology of growth hormone in pregnant women and partial characterisation of the placental GH variant. *J Clin Endocrinol Metab* 1988 (in press).

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Personal practice

Management of prenatally diagnosed uropathies

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It is not unrealistic to anticipate that by the turn of the century virtually every child in the United Kingdom with an appreciable urological abnormality will have been diagnosed by ultrasound before birth. There is a possibility, however, that our ability as clinicians to interpret and utilise information derived from prenatal ultrasound will not keep pace with the increasing sophistication and availability of the imaging techniques. Current management of prenatally diagnosed uropathies is based as much on empiricism as on science. Therapeutic strategies and indications for surgery based on experience with symptomatic conditions in older children are not necessarily relevant to neonates with asymptomatic anomalies diagnosed prenatally.

Prenatal diagnosis: accuracy and reliability

It is important to have realistic expectations of ultrasound. It can provide only anatomical information—which in this context usually means dilatation of the urinary tract. As it cannot reliably distinguish between high grade obstruction, low pressure non-obstructive dilatation or reflux, a final urological diagnosis depends upon the use of cystography and isotopes, which are only available postnatally.

Nevertheless, prenatal ultrasound screening can serve two important roles. The first is in identifying grossly affected fetuses at a time when termination of pregnancy is still an option. The second role lies in its ability to identify fetuses with uropathy that require appropriate investigation in postnatal life.

Within this limited framework prenatal ultrasound is highly effective. With modern real time scanners diagnostic errors are more likely to be due to inexperience in obtaining and interpreting the images rather than any deficiency of the equipment. Some skilled specialist radiologists are now able to provide highly detailed anatomical information—particularly in later pregnancy. While this is desirable (it enables us to give a more accurate prognosis to the parents) it is not essential. At present it is more realistic to regard prenatal ultrasound as a means of screening fetuses for uropathies that will require investigation in postnatal life.

A recent analysis of about 47 000 pregnancies over a five year period in Leeds yielded an incidence of prenatally diagnosed uropathies of 1/570 pregnancies. This figure includes those pregnancies that were terminated and those that subsequently resulted in neonatal death from pulmonary hypoplasia. If these non-viable fetuses are excluded from the calculation, we arrive at a figure of one live born neonate with a significant urological abnormality in every 800 live births. Thus the 'pick-up' rate for prenatal diagnosis is now within the incidence range previously recorded in large postmortem studies. (For example, one study of 245 000 necropsies reported an incidence of renal anomalies of 1/650.[1]) Our figures suggest that prenatal ultrasound diagnosis is highly effective and is already capable of picking up most important urological abnormalities before birth.

Although we can rely increasingly on the ability of ultrasound to detect abnormal kidneys that are dilated, this is not the case with kidneys that are small or absent. We should be very reluctant to advise any action or invasive intervention on the basis of an ultrasound diagnosis of renal agenesis.

Obstetric implications of prenatally diagnosed uropathies

The specialised areas of intrauterine diagnosis and fetal intervention are the joint concern of obstetricians, radiologists, and paediatric specialists. The latter are generally better placed to advise on the likely significance of ultrasound findings and to predict the likely prognosis and course of postnatal management. Perhaps the most effective way of ensuring paediatric involvement in obstetric management is to set up a combined prenatal counselling clinic. In practice, however, it may prove very difficult to reorganise the timetables of the various consultant specialists whose presence at such a clinic is essential.

The gestational age at which routine ultrasound scanning is undertaken is something of a compromise. Most obstetricians settle for the period between 17 and 20 weeks. Scanning before 17 weeks yields less detailed anatomical information and a lower pick up rate for congenital abnormalities. Routine scanning after 20 weeks is more accurate, but leaves less time to acquire additional information on the fetus upon which to base a decision to terminate the pregnancy. Scans undertaken between 17 to 20 weeks can be expected to pick up most of the lethal urological abnormalities. These scans can also yield a great deal of anatomical information: biparietal diameter and fetal length; they can show longitudinal and transverse views of the spine and four chamber views of the heart; and provide information on the stomach, kidneys, and bladder. At this stage in the pregnancy, however, dilatation due to reflux or to less severe forms of obstruction may not yet be evident.

The findings of an appreciable urological abnormality on the initial dating scan should be confirmed by a second examination—ideally performed by a radiologist with particular expertise and experience in prenatal diagnosis. Some form of radiological 'second opinion' is highly desirable if termination of pregnancy is one of the options under consideration. Indeed there is a strong case for referring the mother to a regional centre to be scanned by a radiologist working in conjunction with an obstetrician trained in intervention techniques and a paediatric urologist (or surgeon).

Obstetric intervention can take the form of intrauterine drainage (fetal surgery), termination of pregnancy, or induction of premature labour.

(1) INTRAUTERINE DRAINAGE

Fetal surgery—to drain the obstructed urinary tract—is a logical and, at first sight, attractive extension of prenatal diagnosis. Unfortunately there are several flaws in this concept.

The first is that the renal dysplasia and pulmonary hypoplasia may already be irreversible by the time the urinary tract dilatation is first detected at 17 to 20 weeks. This dilemma is illustrated by the first published case of fetal surgery, which was reported by Harrison and his colleagues in San Francisco.[2] In this case a fetus with severe outflow obstruction was operated upon via a hysterotomy at 21 weeks' gestation. Despite adequate decompression of the urinary tract by cutaneous ureterostomy, the affected neonate died of pulmonary hypoplasia after delivery at 35 weeks. Early fetal lung development appears to depend on an adequate volume of liquor—of which fetal urine is a major constituent. In its analysis of fetal intervention for obstructive uropathy, the International Fetal Surgery Registry reported neonatal death from pulmonary hypoplasia in 27 (37%) of 73 fetuses treated in utero.[3] In a further 11 cases the pregnancy was subsequently terminated. It is highly likely that these fetuses would also have succumbed to pulmonary hypoplasia if the pregnancies had proceeded to term. These figures and those published in a recent review of the literature suggest that intrauterine surgery to drain the urinary tract is unlikely to reduce neonatal mortality from pulmonary hypoplasia.[4]

There is another major concern about intrauterine intervention. Dilatation is not synonymous with obstruction. Ultrasound imaging cannot distinguish between dilatation, which is a consequence of high pressure obstruction (for which it is possible to make a theoretical case of intervention) and non-obstructive dilatation or reflux for which intervention would be inappropriate. Furthermore, fetal intervention has a published procedure related complication rate of 44%[4] and a procedure related mortality rate of anything up to 10%.[3 4] The published data do not show a benefit from fetal intervention that justifies these risks.

Fifty nine fetuses with bilateral uropathy have been managed in Leeds during the last five years. In no instance has intrauterine drainage been attempted. In 15 cases the fetus was severely affected (oligohydramnios, etc, see below) and the pregnancy was terminated. A further eight cases resulted in neonatal death from pulmonary hypoplasia. Thus in this series of fetuses managed without intrauterine drainage, a total of 23 (39%) were non-viable as a result of severe urinary tract obstruction. The mortality reported by the International Fetal Surgery Registry was 59% despite intervention.[3] It would be unwise, however, to draw any firm conclusions from these figures as there may have been important differences between the two groups of fetuses. Long term follow up is essential. Nevertheless our limited experience does not suggest that intrauterine intervention offers any great benefit. It is our view that the continuing use of these procedures can only be justified in the context of a formal prospective controlled trial.

(2) TERMINATION OF PREGNANCY

The most important indicator of poor prognosis is oligohydramnios, but others include 'bright' (dysplastic) renal parenchyma, a high concentration of urinary sodium (greater than 100 mmol/l) on fetal bladder puncture, and evidence of serious coexistent anomalies. The decision to offer termination should be taken on the basis of several scans, ideally performed by more than one radiologist.

(3) INDUCTION OF PREMATURE LABOUR

The theoretical benefits from premature delivery and prompt ex utero surgery have to be set against the additional morbidity associated with prematurity. In our view it is not justifiable to induce premature labour in cases of fetal uropathy, but there may be an argument for allowing premature labour to proceed if it occurs spontaneously after 34 weeks.

Counselling during pregnancy

When fetal uropathies were first diagnosed with relative frequency, there was some debate about the wisdom of informing parents of the abnormal ultrasound findings. It was argued, by some, that this information would provoke unnecessary anxiety during pregnancy. There is no question, however, of the need to inform parents of the presence of a severe uropathy for which termination (or possibly intervention) might be appropriate. The argument centres on whether parents should be told about the less severe anomalies for which no treatment is envisaged until delivery. We believe that parents should be informed as soon as the presence of a fetal uropathy has been confirmed. This information should be followed by appropriate counselling and the opportunity for the parents to meet the specialist who will be supervising their child's management after delivery. Apart from any virtue that an open approach might have, it is difficult, in practice, to conceal from parents the fact that an abnormality has been detected on ultrasound scanning. Most mothers seem to sense that 'something is wrong' and are aware of the unusual level of interest being shown in their scans.

Postnatal management

For practical purposes the value of prenatal diagnosis lies in its ability to identify clinically undetectable urological abnormalities that would otherwise only have come to light in later life as a result of infection or impaired renal function. It is important, however, not to overstate the contribution of prenatal diagnosis to survival or well being. In a review of 145 live born neonates with prenatally diagnosed uropathy, we found that there were 24 (17%) who had relevant physical signs or stigmata of urological disease that rendered the prenatal diagnosis largely irrelevant. In 121 neonates (83%), however, there were no physical signs and the infant's urological abnormality was picked up solely on the basis of information from the prenatal ultrasound scans. In 25 neonates (17%) we felt that the prenatal diagnosis had been of definite value in identifying appreciable treatable pathology affecting both kidneys or a solitary kidney. It is likely that prenatal diagnosis did make a useful contribution to the prognosis for renal function in this group of neonates. In 65 neonates (45%) an appreciable uropathy was present, but was confined to one kidney—the contralateral kidney being healthy. In this group we catagorised the prenatal diagnosis as being of 'probable' value. Finally there was a sizeable group—31 (21%)—with mildly dilated systems of uncertain significance. In most of these cases the dilatation has resolved spontaneously in the first year of life. It is possible that some had reflux in utero. Alternatively, some of these infants may have incipient pelviureteric obstruction, which may yet cause problems in later life. Time will tell.

INVESTIGATIONS

There is little doubt that the initial investigation should be an ultrasound scan of the urinary tract performed within the first 24 or 48 hours of life. Further investigation then flows from the initial ultrasound findings (figure).

(a) Initial postnatal scan normal

There is no evidence of dilatation. The parents are reassured, the infant is discharged from hospital at the normal time, and is brought back to be scanned again at 1 month. If this second scan is also normal we would discharge the child from further follow up.

(b) Initial scan shows mild or equivocal dilatation of the pelvicalyceal system

Although some radiologists regard 1 cm as the upper limit of normal for the diameter of the renal pelvis, this is a somewhat arbitrary figure. Most of these mildly dilated systems are probably normal, but

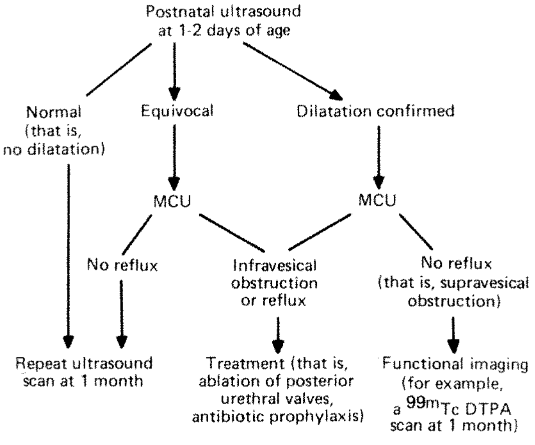
Postnatal ultrasound at 1-2 days of age
Normal (that is, no dilatation)
Equivocal
Dilatation confirmed
MCU
MCU
No reflux
Infravesical obstruction or reflux
No reflux (that is, supravesical obstruction)
Repeat ultrasound scan at 1 month
Treatment (that is, ablation of posterior urethral valves, antibiotic prophylaxis)
Functional imaging (for example, a 99mTc DTPA scan at 1 month)

Figure *Simplified scheme for postnatal evaluation. MCU=micturating cystography.*

within this group there will be some cases of vesicoureteric reflux and some potential pelviureteric junction obstructions. Until recently we have not had a uniform policy for the investigation of these mildly dilated systems. Micturating cystography has been undertaken in some children, but not in others. We are, however, in the process of setting up a prospective study to try to determine whether the frequency of vesicoureteric reflux in this group is sufficient to justify screening of all infants with mildly dilated systems by cystography.

(c) Unequivocal dilatation

The initial scan will not only confirm the presence of dilatation, but should also provide additional information on the likely aetiology—that is, thick walled bladder, urethral valves, ureterocele, etc. We have come round to the view that virtually every neonate with unequivocal dilatation of the urinary tract should undergo a micturating cystogram to show possible bladder outflow obstruction or vesicoureteric reflux.

Functional imaging—for example, intravenous urography, isotope renography—is generally unhelpful and may even be misleading in the period of 'transitional nephrology' in the first month of life. Furthermore these investigations are unlikely to contribute to practical management at this early stage. Unilateral uropathies (which constituted two thirds of our cases) do not constitute a threat in the neonatal period, and functional imaging can reasonably be deferred. Bilateral uropathies are usually the consequence of bladder outflow obstruction, reflux or bilateral pelviureteric junction obstruction. Initial treatment of urethral valves can be undertaken on the basis of the findings on the micturating cystography. Similarly the treatment of reflux (antibiotic prophylaxis) can be instituted without functional imaging of the upper urinary tract. Bilateral pelviureteric junction obstruction may, however, call for an early ^{99m}Tc diethylene triamine pentaacetic (DTPA) scan to plan management and to determine the possible need for percutaneous nephrostomy.

PRACTICAL CONSIDERATIONS

The first practical difficulty may be in identifying affected but asymptomatic neonates on the postnatal wards. We have certainly had experience of infants with significant uropathy who have been examined and discharged by an unsuspecting paediatric senior house officer. It is important that the presence of a prenatally diagnosed uropathy is indicated clearly on the maternal case notes—for example, by use of a sticker on the folder.

The management of prenatally diagnosed uropathies can be effectively undertaken on a shared basis between a consultant paediatrician in a district hospital and a paediatric urologist or paediatric surgeon based in the relevant regional centre. It is likely that the specialist surgeon will already be working in conjunction with a regional paediatric nephrologist and a paediatric radiologist with a particular interest in these problems. Communication is important to avoid unnecessary duplication of investigations.

The aims of postnatal management can be summarised as the preservation of renal function and the prevention of infection. It is becoming clear that obstruction in the absence of infection gives rise to gradual rather than rapid deterioration of renal function in this age group. The tendency of some surgeons to proceed to pyeloplasty in the first few days of life has very little justification. The use of antibiotic prophylaxis in infants with prenatally diagnosed uropathies has not been subjected to a controlled trial. Nevertheless the use of antibiotic prophylaxis in reflux seems a logical extension of experimental and clinical work on reflux nephropathy in older children. The importance of prophylaxis in cases of obstructive uropathy is less clear. Before the advent of prenatal diagnosis urinary infection was the most common form of presentation of pelviureteric junction obstruction in infants under 1 year of age.[5] For this reason we routinely prescribe trimethoprim prophylaxis in this group, at least for the first year of life.

Aspects of surgical management

Indications for surgical intervention that have been derived from experience with symptomatic abnormalities may not be relevant to the management of asymptomatic conditions such as multicystic kidney, mild pelviureteric junction obstruction or reflux diagnosed on prenatal ultrasound scanning. It is important that the management of prenatally diagnosed uropathies evolves on the basis of analysis of results and appropriately controlled studies.

POSTERIOR URETHRAL VALVES

Male infants with urethral valves represent about 10% of the total live born infants with significant prenatally diagnosed uropathies. Posterior urethral valves, however, account for one third of the cases of bilateral uropathy in surviving infants. At present, posterior urethral valves appear to be about the only condition for which fetal surgery (vesicoamniotic shunting) might be of benefit. It is possible, however, that the renal dysplasia seen in this condition may be irreversible by the time it is first detected on ultrasound, and thus not amenable to decompression in utero. A further problem lies in the difficulty in distinguishing urethral valves from other causes of bilateral fetal uropathy such as the prune belly syndrome and primary vesicoureteric reflux. It is possible that the main benefit of prenatal diagnosis in this condition may be in preventing severe urinary infection (often accompanied by septicaemia), which used to be a common presentation in the past.

It seems likely that prenatal diagnosis of posterior urethral valves will improve the prognosis for these boys. Some form of controlled trial of fetal surgery (or early delivery) compared with conventional delivery and prompt ex utero surgery is desirable. Follow up into the teens will be essential if any reliable conclusions are to be drawn from such studies.

At present we do not favour fetal surgery nor premature delivery. Management consists of prompt investigation after delivery and early endoscopic surgery to ablate the valve tissue.

PELVIURETERIC JUNCTION OBSTRUCTION

Obstruction of the pelviureteric junction is the single most frequently diagnosed form of fetal uropathy. The initial surgical response (particularly in the United States) was to proceed to pyeloplasty in the neonatal period. It is becoming clear, however, that some of these pelviureteric obstructions resolve spontaneously in infancy. (Whether they will recur in later life is, of course, unresolved.) Furthermore this form of obstruction appears to be relatively benign and only gradually progressive. Even in the days before prenatal diagnosis, it was unusual to have to remove one of these kidneys because of non-function. It is possible that neonatal pyeloplasty may result in the preservation of a small percentage of the glomerular filtration rate that would otherwise be lost. This theoretical benefit must be offset against the likelihood that some kidneys will be lost or badly damaged by anastamotic obstruction and other complications of this technically difficult surgery.

In general our management of 37 infants (47 obstructed kidneys) has been based on the criteria suggested by Ransley and Manzoni.[6] On the basis of their experience these authors suggested that kidneys that show good preservation of function (greater than 40% of the differential function on an isotope scan) can be managed conservatively even if there is evidence of active obstruction. Conservative management takes the form of antibiotic prophylaxis, serial ultrasound imaging, and further dynamic isotope imaging at 6 to 12 months of age. Evidence of impaired function (less than 40% of differential function) is an indication for early pyeloplasty. In those cases where the kidney is functioning very poorly (less than 20% function) it may be helpful to insert a percutaneous nephrostomy and to reassess function with an isotope scan before deciding between pyeloplasty or nephrectomy.

This conservative approach to the management of prenatally diagnosed pelviureteric obstruction may prove to be misguided. At worst, however, it will lead to the deferment of surgery, in the knowledge that function is most unlikely to deteriorate rapidly. At best it will save some children from undergoing an unnecessary major operation.

MULTICYSTIC KIDNEY

Multicystic dysplastic kidney is a more common anomaly than was previously recognised. In the days before prenatal diagnosis most of these abnormal kidneys almost certainly went unrecognised. A recent analysis of about 47 000 live births yielded an incidence of this condition of around 1/4500 live births.[7] It was common practice to remove those multicystic kidneys that presented clinically because of the possible risks of hypertension and malignant change. In fact these risks appear to be very slight—particularly in relation to the true incidence of the condition. We recently undertook a literature search covering a 20 year period. This identified only three documented cases of hypertension and six cases of malignancy. The risk of complications, therefore, seems to extremely small and for this reason we have abandoned 'prophylactic' nephrectomy. Follow up imaging with ultrasound shows that the multicystic kidneys regress, in fact we have had two that have disappeared completely. It is likely that regression of a multicystic kidney accounts for a substantial proportion of those cases of apparent unilateral renal agenesis.

The main importance of multicystic kidney is as an indicator of coexistent urological abnormalities. These should be sought by appropriate investigations.

VESICOURETERIC REFLUX

Prenatal diagnosis offers an exciting opportunity to study the natural history of vesicoureteric reflux and of reflux nephropathy. Several interesting features are already emerging. Reflux is generally viewed as a disorder of females. In our series of 22 cases of prenatally diagnosed reflux, however, only three were girls. This suggests that asymptomatic reflux is more common in boys than is generally recognised. It is widely believed that reflux nephropathy results from the combination of reflux (particularly intrarenal reflux) and urinary infection occurring in infancy. If this view is correct, then the prenatal diagnosis of reflux should result in a reduction of scarring and its associated morbidity by ensuring that affected children are started on antibiotic prophylaxis from the first day of life. Our limited results so far suggest that this may indeed be the case. The interpretation of these results, however, is complicated by the high proportion of boys—many of whom might have escaped infection and scarring even if they had not been managed by antibiotic prophylaxis.

Reimplantation surgery is best avoided in the infant. Not only is it technically difficult and less successful, but in the context of prenatally diagnosed reflux, it is usually unnecessary.

Prenatal urological diagnosis will inevitably lead to increasing surgical specialisation. There can be very little justification for anyone other than a suitably trained paediatric urologist or surgeon operating upon the urinary tract of a small infant. This contrasts with the 'medical' aspects of management (checking for urinary infection, blood pressure measurements, biochemical investigations, and general follow up) that are best undertaken in paediatric departments in district hospitals.

Communication and cooperation will prove essential if we are to utilise prenatal diagnosis for the maximum benefit of the children under our care.

References

[1] Ashley DJB, Mostofi FK. Renal agenesis and dysgenesis. *J Urol* 1960;**83**:211–30.
[2] Harrison MR, Golbus MS, Filly RA, *et al.* Fetal surgery for congenital hydronephrosis. *N Engl J Med* 1982;**306**:591–3.
[3] Manning FA, Harrison MR, Rodeck C, *et al.* Catheter shunts for fetal hydronephrosis and hydrocephalus. *N Engl J Med* 1986;**315**:336–40.
[4] Elder JS, Duckett JW, Snyder HM. Intervention for fetal obstructive uropathy: has it been effective? *Lancet* 1987; **ii**:1007–10.
[5] Thomas DFM, Agrawal M, Laidin AZ, Eckstein HB. Pelvi ureteric obstruction in infancy and childhood. A review of 117 patients. *Br J Urol* 1982;**54**:204–8.
[6] Ransley PG, Manzoni G. Extended role of DTPA scan in assessing function and PUJ obstruction in neonates. *Dialogues in Paediatric Urology* 1985;**8**:6–8.
[7] Gordon AC, Thomas DFM, Arthur RJ, Irving HC. Multicystic kidney—is nephrectomy still appropriate? *J Urol* (in press).

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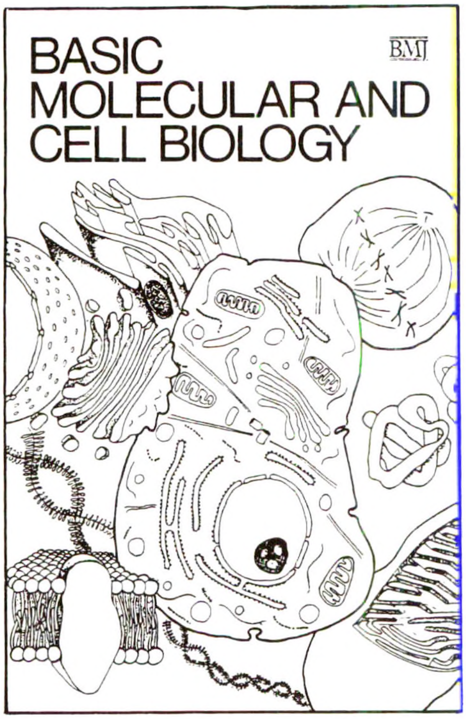

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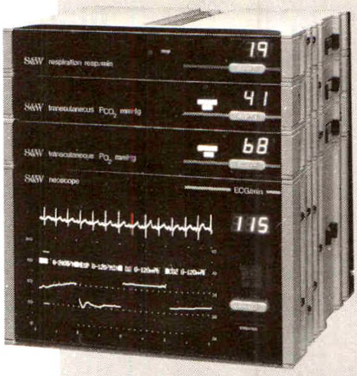

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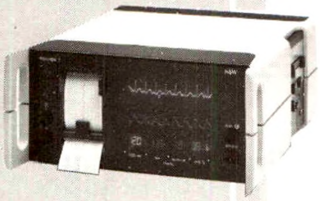

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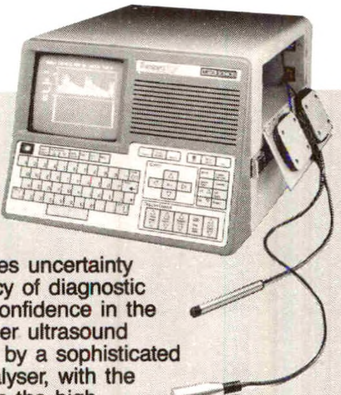

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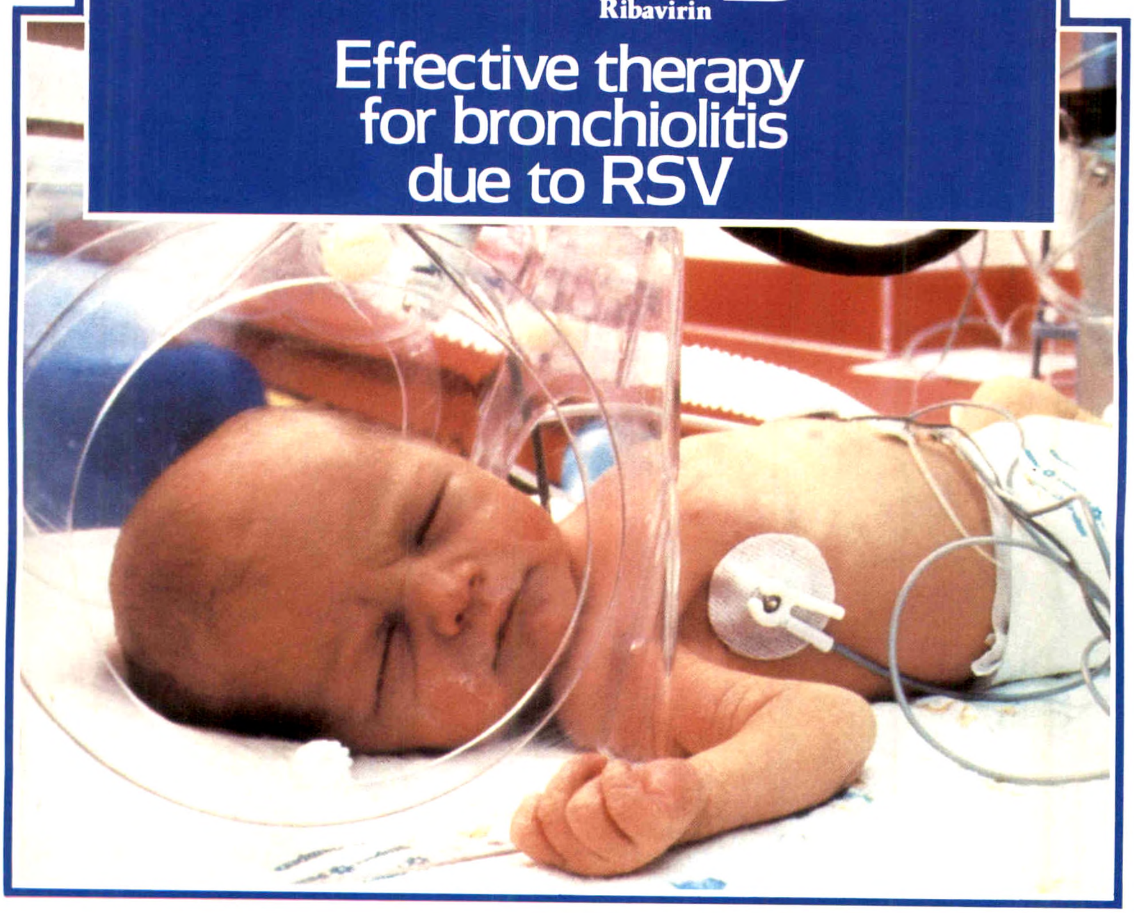

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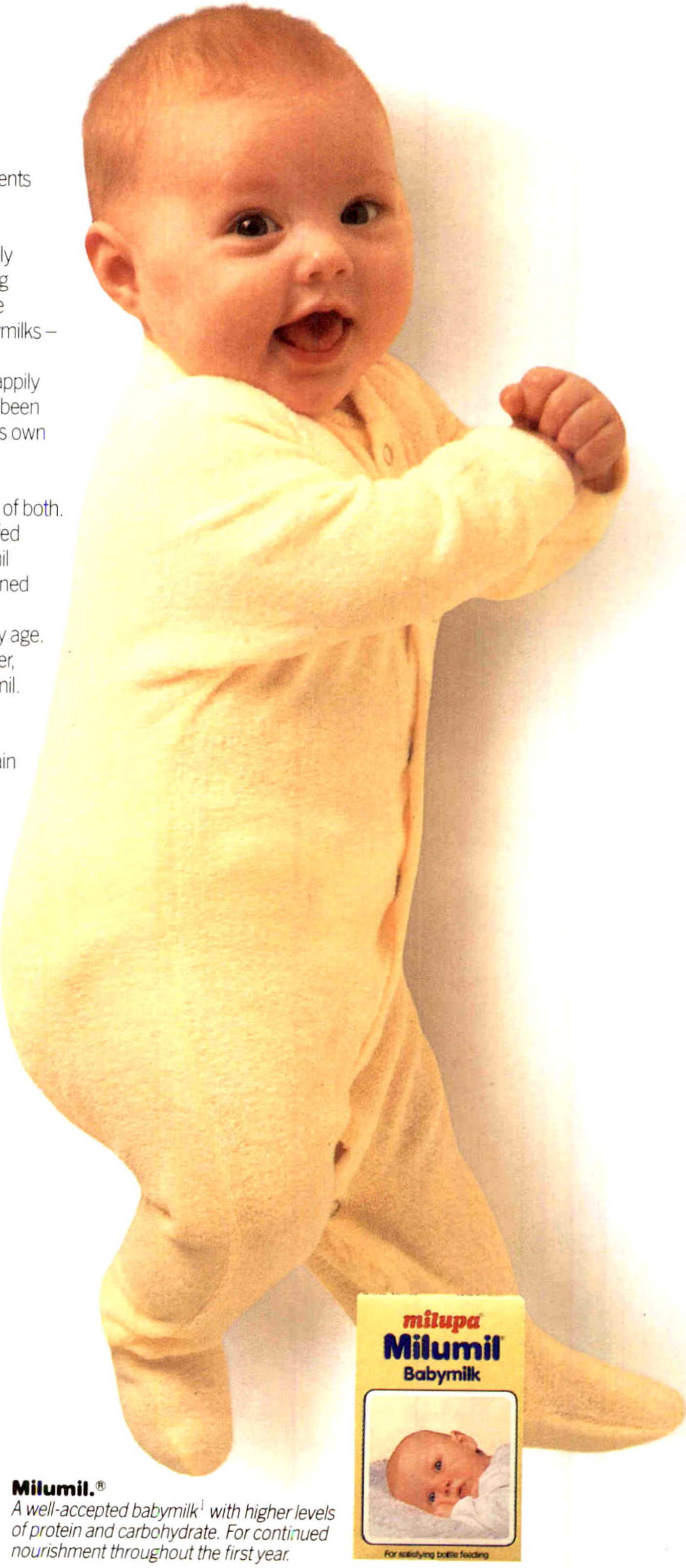

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Annotations

Pain and analgesia in the newborn

The traditional view that neonates do not feel pain is now being questioned. Early studies showed what was thought to be a decorticate response in babies with no localisation to painful stimuli and this, together with the belief that the painful experience was not remembered, implied that pain was unimportant.[1] As a consequence and because of certain anxieties about the handling of drugs (particularly opiates) in the newborn there has been a reluctance to prescribe analgesia for these patients.[2] Data now suggest that infants do feel pain.[1 3 4] In this review the underlying neurobiology of pain development in neonates is described, followed by a review of clinical studies indicating that the newborn baby responds (possibly adversely) to pain. We conclude that the arguments in favour of the use of analgesia have become overwhelming.

Neurobiology of pain development

The limited evidence available shows that innervation of the peripheral tissue and the basic connections between primary sensory neurones and the cells in the dorsal horn of the spinal cord occurs early in fetal development. In this review we concentrate on those events that seem to occur later in fetal life or in the early postnatal period. In rats the central neurophysiological effects of unmyelinated C fibres, the group of afferents that are particularly concerned in chronic and inflammatory pain, mature gradually over the first two to three postnatal weeks.[5 6] This maturation is associated with several anatomical and chemical changes. The concentrations of neuropeptides found within the C fibres (such as substance P, somatostatin, and vasoactive intestinal polypeptide) increase considerably during the early postnatal period.[7] Furthermore, at birth binding sites for substance P are in high concentrations and distributed diffusely over the grey matter, and only become defined in specific laminae and reduced in number as the rat matures.[8] Whether there is a similar delayed development of C fibres in man is not known, but there is a pronounced increase in the concentrations of the above neuropeptides around the time of birth.[7 9]

The activity of interneuronal pathways in the dorsal horn, especially relevant to pain processing, also develops postnatally. The spinal cord lacks inhibitory control at birth and although the synaptic linkage between afferents and dorsal horn cells is still weak, receptive fields are large and single stimuli can often evoke long lasting excitation that lasts for several minutes; repeated stimuli can build up considerable background activity in the cells.[5] The result is that otherwise weak cutaneous inputs are made more effective centrally. The flexor reflex, a useful measure of nociceptive function at the spinal cord level, is highly exaggerated in neonates.[10 11] Thresholds are lower and the flexion withdrawal lasts longer. Repeated skin stimulation results in considerable hyperexcitability or sensitisation, which is particularly pronounced before 35 weeks' gestation in the human and the eighth postnatal day in rats.[11] Nevertheless there is a marked reduction in the threshold of this reflex after skin injury in the newborn that is analogous to the tenderness or hyperalgesia experienced in adults.[12] Inhibitory pathways descending from the brain stem are ineffective at birth,[13] perhaps because their neurotransmitters (5-hydroxytryptamine and noradrenaline) appear only postnatally.[14]

Development of opioid sensitivity

Another important postnatal event is the maturation of the opioid/opiate system in the spinal cord. No analgesic effects of morphine can be detected in the rat pup until the seventh postnatal day and adult sensitivity is only reached at the 14th postnatal day, exactly coinciding with the appearance of high affinity mu-receptor binding in the rat cord. K-receptors and ketocylazocine analgesia mature earlier at the seventh to 10th postnatal days.[15] Delta receptors develop last at the 12th postnatal day but the mu:delta ratio does not reach 1:1 until the 26th postnatal day.[16] Little is known about opiate receptor development in the human cord, but it apparently begins before birth. Morphine and other opioids are effective analgesics in newborn infants born both prematurely and at full term, although dose response studies have not been undertaken.[1 4] Enkephalin appears postnatally (first postnatal day) in the rat and is found later than most other peptides in the human fetal dorsal horn at 12–14 weeks.[17]

Assessment of pain in the newborn infant

Three types of response to painful stimuli have been studied in the newborn human infant.

AUTONOMIC RESPONSES

Changes in heart rate, blood pressure, transcutaneous partial pressure oxygen, and palmar sweating are common autonomic responses to pain. In newborn babies, lancing of the heel produces sustained increases in heart rate in full term infants but the responses are less clear in the preterm baby.[18] The palmar sweating response to this stimulus does not appear until 37 weeks' gestation.[19] Local anaesthesia prevents the heart rate and blood pressure changes associated with circumcision.[20]

BEHAVIOURAL RESPONSES

Diffuse body movement and purposeful withdrawal have been quantified in response to painful stimuli[21] and have been used to show that the preterm infant is, if anything, supersensitive to painful stimuli when compared with the full term infant.[11] These responses can be reduced by local analgesia (M Fitzgerald, C Millard, N McIntosh, unpublished observations). Facial expressions have also been linked with painful stimuli but vary with the behavioural state.[22] Crying (? primitive communication) has been evaluated clinically and by spectrographic analysis. A painful cry is different from that indicating hunger or fear in a full term baby, but the differentiation is difficult in the preterm infant.[23]

HUMORAL AND METABOLIC RESPONSES

Anand *et al* studied the response to surgical operations in neonates.[24] A pronounced release of catecholamines, growth hormone, glucagon, aldosterone, and other corticosteroids was seen when insulin secretion was suppressed. These responses were associated with the breakdown of fat and the release of carbohydrates resulting in increased concentrations of glucose, lactate, pyruvate, ketone bodies, and non-esterified fatty acids in the blood, as well as an increase in the breakdown of protein.[4] Potent anaesthetic agents (for example, fentanyl and halothane) inhibited these stress responses[25] and preliminary data suggested that the stress response might be associated with increased postoperative morbidity and mortality.[14]

Conclusions

Laboratory studies have shown that the basic connections required for painful stimuli to be transmitted to the central nervous system are present in the newborn, despite the considerable organisation and maturation (particularly of control systems) that occurs postnatally. Clinical studies of the pain responses mounted by newborn babies have confirmed that these pathways are functional, albeit somewhat variable. Furthermore, in some cases these behavioural changes persisted after the painful procedure, implying that the experience was remembered.[26] This behaviour may disrupt both the infants' adaptive responses to feeding and the mother/infant reactions.[27] Anaesthetic agents reduce the stress response after neonatal operations. It is difficult at this stage to evaluate whether a profound stress response is good or bad. Stability of physiology is important in the preterm infant: fluctuations of arterial oxygen pressure may be potentially dangerous to the eye,[28] and variability in blood pressure has been linked with the occurrence and extension of intraventricular haemorrhage.[29] The suggestion, however, that there are fewer side effects of operation when potent anaesthetic agents are used,[24] and that postoperative mortality and morbidity are reduced, mean that the judicious provision of analgesia is important, independent of whether the pain is remembered.

References

[1] McGrath PJ, Unruh AM, eds. *Pain in children and adolescents. Pain research and clinical management.* Vol 1. Amsterdam: Elsevier, 1987.

[2] Purcell-Jones G, Dormon F, Sumner E. Paediatric anaesthetists' perceptions of neonatal and infant pain. *Pain* 1988;**33**:181–7.

[3] Fitzgerald M. Pain and analgesia in neonates. *Trends in Neuroscience* 1987;**10**:344–6.

[4] Anand KJS, Hickey PR. Pain and its effects in the human neonate and fetus. *N Engl J Med* 1987;**317**:1321–9.

[5] Fitzgerald M. The postnatal development of cutaneous afferent fibre input and receptive field organization in the rat dorsal horn. *J Physiol* 1985;**364**:1–18.

[6] Fitzgerald M. The development of activity evoked by fine diameter cutaneous fibres in the spinal cord of the newborn rat. *Neurosci Lett* 1988;**86**:161–6.

[7] Marti E, Gibson SJ, Polak JM, *et al.* Ontogeny of peptide and amino-containing neurons in motor, sensory and autonomic regions of rat and human spinal cord. *J Comp Neurol* 1987;**266**: 332–59.

[8] Charlton CG, Helke CJ. Ontogeny of substance P receptors in the rat spinal cord: quantitative changes in receptor number and differential expression in specific loci. *Developmental Brain Research* 1986;**29**:81–6.

[9] Charnay Y, Chayvialle JA, Pradayrol L, Bouvier R, Paulin C, Dubois PM. Ontogeny of somatostatin-like immunoreactivity in the human foetus and spinal cord. *Developmental Brain Research* 1987;**36**:63–73.

[10] Ekholm J. Postnatal changes in cutaneous reflexes and in the discharge pattern of cutaneous are articular sense organs. *Acta Physiol Scand* 1967;**297**(suppl 7):1–130.

[11] Fitzgerald M, Shaw A, McIntosh N. Postnatal development of the cutaneous flexor reflex: comparative study of preterm infant and newborn rat pups. *Dev Med Child Neurol* 1988;**30**:520–6.

[12] Fitzgerald M, Millard C, McIntosh N. Hyperalgesia in premature infants. *Lancet* 1988;**i**:292.

[13] Fitzgerald M, Koltzenburg M. The functional development of descending inhibitory pathways in the dorsolateral funiculus of

the newborn rat spinal cord. *Developmental Brain Research* 1986;**24:**261–70.

[14] Bregman BS. Development of serotonin immunoreactivity in the rat spinal cord and its plasticity after neonatal spinal cord lesions. *Developmental Brain Research* 1987;**34:**245–64.

[15] Barr GA, Paredes W, Erickson KL, Zukin RS. K-opioid receptor-mediated analgesia in the developing rat. *Developmental Brain Research* 1986;**29:**145–52.

[16] Wohltmann M, Roth BL, Coscia CJ. Differential postnatal development of mu and delta opiate receptors. *Developmental Brain Research* 1982;**3:**679–84.

[17] Charnay Y, Paulin C, Dray F, Dubois PM. Distribution of enkephalin in human fetus and infant spinal cord: an immunofluorescence study. *J Comp Neurol* 1984;**223:**415–23.

[18] Owens ME, Todt EH. Pain in infancy: neonatal reaction to a heel lance. *Pain* 1984;**20:**77–86.

[19] Harpin CA, Rutter N. Development of emotional sweating in the newborn infant. *Arch Dis Child* 1982;**57:**691–5.

[20] Williamson PS, Williamson ML. Physiological stress reduction by a local anesthetic during newborn circumcision. *Pediatrics* 1983;**71:**36–40.

[21] Frank LS. A new method to quantitatively describe pain behaviour in infants. *Nurs Res* 1986;**35:**28–31.

[22] Grunau RVE, Craig KD. Pain expression in neonates: facial action and cry. *Pain* 1987;**28:**395–410.

[23] Michelsson K, Jarvenpaa A-L, Rinne A. Sound spectrographic analysis of pain cry in preterm infants. *Early Hum Dev* 1983;**8:**141–9.

[24] Anand KJS, Brown MJ, Causon RC, Christofides ND, Bloom SR, Aynsley-Green A. Can the human neonate mount an endocrine and metabolic response to surgery? *J Pediatr Surg* 1985;**20:**41–8.

[25] Anand KJS, Sippell WG, Aynsley-Green A. Randomised trial of fentanyl anaesthesia in preterm neonates undergoing surgery: effects on the stress response. *Lancet* 1987;**i:**243–8

[26] Marshall RE, Stratton WC, Moore JA, Boxerman SB. Circumcision. I. Effects upon newborn behaviour. *Infant Behaviour and Development* 1980;**3:**1–14.

[27] Marshall RE, Porter FL, Rogers AG, Moore JA, Anderson B, Boxerman SB. Circumcision. II. Effects upon mother-infant interaction. *Early Hum Dev* 1982;**7:**367–74.

[28] Venus V, Patel KC, Pratap KS, Konchigeri H, Vidyasagar D. Transcutaneous PO_2 monitoring during pediatric surgery. *Crit Care Med* 1981;**9:**714–16.

[29] Goodard J, Lewis RM, Armstrong DL, Zeller RA. Moderate rapidly induced hypertension as a cause of intraventricular hemorrhage in the newborn beagle model. *J Pediatr* 1980;**96:**1057–60.

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Blood pressure monitoring in the newborn

The association of cerebral palsy and cerebral ischaemic lesions such as periventricular leucomalacia is now clearly established.[1-4] Such lesions are likely to be caused by changes in cerebral perfusion. The hypothesis that cerebral perfusion is 'pressure-passive' in some newborn infants is becoming increasingly widely held and in these infants changes in systemic blood pressure are likely to be directly transmitted to the cerebral circulation.

If we accept this point of view, and there is increasing evidence that we should,[5-7] it is clear that close monitoring of blood pressure is a prerequisite for successful neonatal intensive care. But important questions arise: what method is best? what is the normal range of blood pressure? The purpose of this annotation is to provide answers.

Methods for measuring blood pressure

The requirements for successful blood pressure monitoring are that the method should be easy to set up, reliable, and give continuous information or enable measurements to be made at frequent intervals with minimal disturbance to the baby. Non-invasive and invasive methods are available.

NON-INVASIVE METHODS

The four methods available are the traditional mercury sphygmomanometer and three automatic methods: the Arteriosonde (a Doppler technique), the Infrasonde, and the oscillometric monitor (such as the Dinamap (Critikon)). Each of these methods has serious shortcomings. Traditional flush techniques and methods relying on palpation and auscultation are not sufficiently accurate and just not practical for the small sick infant.[8] Of the three automatic devices available, only the oscillometric type is now in widespread use.

The Doppler method (Arteriosonde) involved the use of a fairly large and clumsy transducer which was placed under a cuff and over the brachial artery. In a neonatal study a correlation coefficient of no better than 0·80 was obtained when compared with measurements from an arterial catheter.[9] This technique has also caused a transient radial nerve palsy in small infants due to pressure by the transducer on the radial nerve which accompanies the brachial artery along the inner aspect of the humerus.[10] The cumbersomeness of this technique has undoubtedly contributed to its failure to gain wide popularity.

The Infrasonde derived its signal in the following way. When an inflated cuff was placed around the limb and deflated, blood passed from the vessel segment under the deflating cuff into the distal portion of artery which was collapsed. This collapsed section fluttered as blood passed into it and this fluttering gave rise to low frequency (infrasonic) vibrations that disappeared when the cuff pressure fell below the diastolic pressure. In a study of children aged between 4 days and 14 years, this device was compared with simultaneous intra-arterial measurements: the authors concluded that although systolic measurements were as good as those obtained by a mercury sphygmomanometer, diastolic measurements were not.[11] I am not aware of any good studies to correlate this technique with invasive blood pressure measurements in very small infants. One problem was that the error in the method was variable—that is, not systematic.

The most popular of the indirect methods is currently the Dinamap oscillometric monitor. The basis for this device is that the arterial wall oscillates when blood flows in pulsatile fashion through a vessel. These oscillations are transmitted to a cuff placed around the limb. As the pressure within the cuff is reduced, the pattern of oscillations changes. When arterial pressure is just above the cuff pressure there is a rapid increase in the amplitude of the oscillations and this is taken as systolic pressure. The point at which the amplitude of the oscillations is maximal coincides with the mean arterial pressure. Diastolic pressure is recorded when there is a sudden decrease in oscillations.[12]

The oscillometric method has serious problems associated with it. Firstly, the regular automatic inflation of the cuff disturbs infants whose clinical condition is unstable and who are being handled as little as possible. Secondly, although several studies of newborn infants have reported the method to be reliable and accurate,[8] the most telling observations have been made by Diprose and colleagues.[13] These authors studied very low birthweight infants and showed that the Dinamap consistently over read systolic and diastolic blood pressures when the infants were hypotensive. Thus the instrument became most unreliable when accurate measurement was of the greatest importance— and there is no reason to believe that other oscillometric monitors perform any better.

The oscillometric method has been enthusiastically promoted. Its popularity is mainly due to its convenience and ease of use. As stated above, however, it is inaccurate when sick infants of very low birth weight are being monitored and this caveat must be borne in mind during neonatal intensive care.

INVASIVE MEASUREMENT

The most widely used method is by means of an umbilical arterial catheter connected to a blood pressure transducer by rigid low compliance pressure tubing. Insertion of the catheter is relatively straight forward. The precise method of insertion is a matter of personal preferences and techniques of insertion and fixation of the catheter are well described in standard neonatal texts.[14]

Some theoretical considerations

There are some theoretical considerations which are of practical importance. The pressure, P, measured by the transducer has three components:

$$P=P_s+P_k+P_r$$

where P_s is the static component, P_k is the kinetic component, and P_r is the reference level component.[15]

When a catheter is introduced into the lumen of a vessel, the observer wants to know what the pressure at the tip of the catheter is. If there is no flow the pressure will be the same in all directions. This is the static component, P_s, and the direction in which the catheter opens (that is, whether it has a hole at the end or at the side) is of no importance.

When flow occurs the blood develops kinetic energy, giving rise to a kinetic pressure component, P_k. The pressure sensed (P_s+P_k) will vary according to the relationship of the catheter opening to the direction of flow. If an end hole catheter is used, the kinetic energy effect is maximal because flow is straight into the catheter. The size of the kinetic energy component ($\frac{1}{2}d.v^2$, where d is the density of blood and v is the velocity) is given by Bernoulli's theorem and is proportional to the square of the blood velocity. Assuming a high mean aortic blood velocity of 40 cm/second, the size of this kinetic pressure component is only 0·6 mm Hg, which is less than 2% of the total mean pressure. During systole, the flow velocity increases to a peak which is approximately three times the mean and P_k increases to 5·7 mm Hg or about 10% of the total systolic pressure. Similarly, P_k will also increase as cardiac output increases. A side hole catheter—where the opening of the catheter is at right angles to the direction of flow—minimises this artifact. Against this theoretical advantage of a side hole catheter, however, must be set the more practical one that these catheters tend to become blocked by blood clot more quickly than end hole catheters.

The reference level component, P_r, is due to the difference in height between the two ends of the catheter. P_r will be zero if the transducer is placed at the same level as the catheter opening.

A further theoretical consideration when selecting a transducer is to choose one with a frequency response which will provide a reasonable representation of the arterial pressure wave. The number of harmonics required relate to the shape of the signal and the accuracy required. For arterial pressure, reproduction using at least seven, and preferably 10, harmonics will give sufficient accuracy.[15] The fundamental frequency of the blood pressure wave is the pulse rate, which is less than 4 Hz, and an arterial pressure transducer for neonatal use has therefore to be able to record at 4 Hz×7=28 Hz. This may be compared with a system intended for adult use, which has to achieve only half of this frequency response.

Two sources of error are particularly noteworthy. A catheter of too small diameter will act as a low pass filter with loss of higher frequencies resulting in under reading particularly of systolic pressures. Another frequent and substantial source of error is the introduction of small air bubbles. Their effects may be explained in terms of the concepts of resonant frequency, underdamping, and overdamping. A perfect transducer should represent a perfectly sinusoidal pressure waveform of any frequency. As with all mechanical systems, a catheter transducer system will resonate at a particular frequency, that is, the amplitude of a measured waveform of that frequency will be selectively increased rather than being truly represented. If a system is to be useful for the practical measurement of arterial blood pressure, the resonant frequency should be as high as possible and beyond the range of frequencies of interest. Thus in the above example, it should be several times greater than 28 Hz. If overdamping or underdamping or resonance occurs, the high harmonics of the signal will be most affected and there will be greater error in the measurement of systolic and diastolic pressures than in the measurement of mean arterial pressure.[13] In a resonating system, systolic and diastolic pressures will be too high and too low respectively. The effect of an underdamped system will be the same,[16] whereas an overdamped system will result in the systolic reading being too low and the diastolic reading being too high. This constitutes another reason for clinicians placing more weight on the mean than on systolic or diastolic pressures.

From a practical point of view, the dicrotic notch on the downward slope of the waveform on the

Table *Mean blood pressure, birth weight, and postnatal age. Results (in mm Hg) are given as 10th percentile (mean)**

Birth weight (g)	*Postnatal age (hours)*								
	3	*12*	*24*	*36*	*48*	*60*	*72*	*84*	*96*
500	23 (35)	24 (36)	25 (37)	26 (38)	28 (39)	29 (41)	30 (42)	31 (43)	33 (44)
600	24 (35)	25 (36)	26 (37)	27 (39)	28 (40)	29 (41)	31 (42)	32 (44)	33 (45)
700	24 (36)	25 (37)	26 (38)	28 (39)	29 (42)	30 (42)	31 (43)	32 (44)	34 (45)
800	25 (36)	26 (37)	27 (39)	28 (40)	29 (41)	31 (42)	32 (44)	33 (45)	34 (46)
900	25 (37)	26 (38)	27 (39)	29 (40)	30 (42)	31 (43)	32 (44)	34 (45)	35 (47)
1000	26 (38)	27 (39)	28 (40)	29 (41)	31 (42)	32 (43)	33 (45)	34 (46)	35 (47)
1100	27 (38)	27 (39)	29 (40)	30 (42)	31 (43)	32 (44)	34 (45)	35 (46)	36 (48)
1200	27 (39)	28 (40)	29 (41)	30 (42)	32 (43)	33 (45)	34 (46)	35 (47)	37 (48)
1300	28 (39)	29 (40)	30 (41)	31 (43)	32 (44)	33 (45)	35 (46)	36 (48)	37 (49)
1400	28 (40)	29 (41)	30 (42)	32 (43)	33 (44)	34 (46)	35 (47)	36 (48)	38 (49)
1500	29 (40)	30 (42)	31 (43)	32 (44)	33 (45)	35 (46)	36 (48)	37 (49)	38 (50)

*Data obtained by birthweight specific regression for mean arterial pressure on postnatal age using data from 131 infants.[18]

visual display unit is most sensitive to changes in the higher harmonics. If the dicrotic notch becomes distorted—it is usually absent when overdamping occurs—the presence of an air bubble in the catheter transducer system should be suspected.

Some other practical considerations

There are some contentious areas: should the tip of the catheter be in a mid-thoracic position or below the origins of the renal arteries? How long should a catheter be left in situ? What are the indications for its removal?

A straw poll of 26 neonatal intensive care units in the United Kingdom showed that 18 favoured a high position whereas five preferred the catheter tip to be below L2, the level of the origins of the renal arteries, two used a high position for infants below 1000 g and a low position for infants above 1000 g, and one centre was conducting a randomised trial to compare the two positions. At Liverpool Maternity Hospital the tip of the catheter is usually sited just above the level of the diaphragm—unless the radiograph performed to check its position shows it to be in the abdominal aorta, in which case it is pulled back to L3/4. Our only absolute indication for catheter removal is ischaemia of the lower limbs or buttocks which does not improve after infusion of plasma. We have no set time limit for removal of a catheter.

Measurements of blood pressure from umbilical arterial catheters have been shown to correlate well with those measured from peripheral arteries.[17]

There are transducers that have pressure domes for multiple use and that are able to withstand chemical sterilisation a number of times. Because this is awkward and time consuming, disposable domes have increased in popularity. Nowadays completely disposable transducers are available. These may be packed with a complete set of lines and flushing devices and are to be recommended for those nurseries where blood pressure monitoring is only undertaken intermittently and where the prospect of assembling a complicated reuseable system may be daunting.

Normal ranges of blood pressure

There is a paucity of data for the very low birthweight infant. Blood pressure data from 131 infants who had peripheral or umbilical arterial lines with end holes and who were admitted to Mersey regional neonatal intensive care unit have been recently collected.[18] Birthweight specific regression for mean arterial pressure on postnatal age was calculated using data from survivors unaffected by drugs. These data are reproduced in the table. The 10th percentile in this study was similar to previously published normal data for blood pressure in infants below 1000 g.[19]

Conclusions

Close monitoring of blood pressure and the correction of hypotension have become an important and essential part of modern neonatal intensive care. Non-invasive techniques for blood pressure measurement are particularly unreliable in small sick infants. Such children should have their blood pressure monitored by means of a transducer attached to an intra-arterial catheter wherever possible. The measurement of mean arterial pressure is less prone to error than systolic or diastolic pressures.

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References

[1] Weindling AM, Rochefort MJ, Calvert SA, Fox TF, Wilkinson A. Development of cerebral palsy after ultrasonographic detection of periventricular cysts in the newborn. *Dev Med Child Neurol* 1985;**27:**800–6.

[2] Stewart A, Reynolds EDR, Hope PL, *et al*. Probability of neurodevelopmental disorders estimated from ultrasound appearance of brains of very preterm infants. *Dev Med Child Neurol* 1987;**29:**3–11.

[3] Graham M, Levene MI, Trounce JQ, Rutter N. Prediction of cerebral palsy in very low birthweight infants: prospective ultrasound study. *Lancet* 1987;**ii:**593–6.

[4] Cooke RWI. Early and late cranial ultrasonographic appearances and outcome in very low birthweight infants. *Arch Dis Child* 1987;**62:**931–7.

[5] Miall-Allen VM, De Vries LS, Whitelaw AGL. Mean arterial blood pressure and neonatal cerebral lesions. *Arch Dis Child* 1987;**62:**1068–9.

[6] Perlman JM, McMenamin JB, Volpe JJ. Fluctuating cerebral blood flow velocity in respiratory distress syndrome. *N Engl J Med* 1983;**309:**204–9.

[7] Rennie JM. Cerebral blood flow velocity variability following cardiovascular support in premature babies. *Arch Dis Child* 1989 (in press).

[8] Kimble KJ, Darnall RA, Yelderman M, Ariagno RL, Ream AK. An automated oscillometric technique for estimating mean arterial pressure in critically ill newborns. *Anaesthesiology* 1981;**54:**423–5.

[9] Dweck HS, Reynolds DW, Cassady G. Indirect blood pressure measurement in newborns. *Am J Dis Child* 1974;**127:**492–4.

[10] Tollner U, Bechinger D, Pohlandt F. Radial nerve palsy in a premature infant following long-term measurement of blood pressure. *J Pediatr* 1980;**96:**921–2.

[11] Savage JM, Dillon MS, Taylor JFN. Critical evaluation and comparison of the Infrasonde, Arteriosonde, and mercury sphygmomanometer in measurement of blood pressure in children. *Arch Dis Child* 1979;**54:**184–9.

[12] Park MK, Menard SM. Accuracy of blood pressure measurement by the Dinamap monitor in infants and children. *Pediatrics* 1987;**79:**907–14.

[13] Diprose GK, Evans DH, Archer LNS, Levene MI. Dinamap fails to detect hypotension in very low birthweight babies. *Arch Dis Child* 1986;**61:**771–3.

[14] Roberton NRC, ed. *Textbook of neonatology*. Edinburgh: Churchill Livingstone, 1986.

[15] Anderson HR, Bergsten O. *Blood pressure. Measurements and methods*. Hersteduang, Denmark: S and W Medico Teknik A/S, 1982.

[16] Garner RM. Direct blood pressure management—dynamic response requirements. *Anesthesiology* 1981;**54:**227–36.

[17] Butt WW, Whyte HW. Blood pressure monitoring in neonates: comparison of umbilical and peripheral artery catheter measurements. *J Pediatr* 1984;**105:**630–2.

[18] Watkins AMC, West CR, Cooke RWI. Blood pressure and cerebral haemorrhage and ischaemia in very low birthweight infants. *Early Hum Dev* 1988 (in press).

[19] Versmold HT, Kitterman JA, Phibbs RH, Gregory GA, Tooley WH. Aortic blood pressure during the first 12 hours of life in infants with birthweight 610 to 4222 grams. *Pediatrics* 1981;**67:**607–13.

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Original articles

The changing pattern of chronic lung disease of prematurity

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SUMMARY Thirty four infants with chronic lung disease of prematurity were reviewed and divided into two groups on the appearance of the chest radiograph. Type 1 disease (n=19) was defined as homogeneous or patchy ill defined opacification in the lungs without coarse reticulation, and type 2 disease (n=11) had the classical appearance of bronchopulmonary dysplasia. Four patients could not be classified. We conclude that type 1 disease represents typical chronic lung disease of premature infants, and type 2 is usually complicated by pulmonary interstitial emphysema. As no infant with type 1 disease died, its histopathological basis is unknown.

Improvements in the care of premature infants, particularly sophisticated ventilation and monitoring techniques, has lead to increasing numbers of babies surviving, even as young as 24 weeks' gestation. Many of these premature infants develop hyaline membrane disease within the first few hours of birth with the associated range of symptoms and signs of the respiratory distress syndrome. Uncomplicated hyaline membrane disease is self limiting and resolves in seven to 10 days; the lungs of the infants who die of other causes after that time show either no membranes or only fragments.[1] Most infants with the respiratory distress syndrome require supplemental oxygen and mechanical ventilation.

Northway *et al* described a chronic lung disease in premature infants that was superimposed on the healing phase of hyaline membrane disease,[2] which they called bronchopulmonary dysplasia, and in the definition of this disease an abnormal chest radiograph was a crucial component. The abnormalities in established bronchopulmonary dysplasia consisted of overexpanded lungs with a coarse reticulation of air cysts and intervening septa.

It is well known that the classical description of bronchopulmonary dysplasia is not standard, and other authors have described a different radiographic pattern of more homogeneous lung opacities.[3 4] As these seem to be milder forms of chronic pulmonary disease opportunities for histopathological confirmation that it is a type of bronchopulmonary dysplasia are infrequent, but Edwards confirmed the diagnosis in two such cases.[5] Tooley diagnosed bronchopulmonary dysplasia in any premature infant who at the age of 30 days still required supplemental oxygen and who also had a pulmonary parenchymal radiographic abnormality.[6] We too have noticed these two distinct types of bronchopulmonary dysplasia, and therefore undertook a review of a cohort of infants born during a three year period to see if our diagnosis was confirmed.

Patients and methods

All cases of chronic lung disease of prematurity in the special care baby unit from 1983 to 1985 inclusive were reviewed. The criteria for inclusion (similar to those of Tooley[6]) were as follows: any premature infant who required mechanical ventilation soon after birth, required supplemental oxygen for at least 28 days, had an abnormal chest radiograph on the 28th day of life, and had no other severe congenital abnormality.

We also recorded birth weight, length of gestation, indications for mechanical ventilation, number of days that intubation and supplemental oxygen were required, the presence of patency of the ductus arteriosus and whether there was evidence of air leaks. Other clinical events that might have affected the radiographic appearances were also noted.

We attempted to divide the radiographic pattern into two types; type 1 consisted of bilateral, ill defined pulmonary opacities that were predominantly perihilar and homogeneous but sometimes patchy. Often these were no more than a haze of density that was fixed and persistent, unchanged by treatment with antibiotics or diuretics, and lacking any coarse reticulation (fig 1).

Type 2 consisted of a coarse reticulation characterised by streaky densities interspersed with small cystic translucencies (fig 2). The translucencies were sometimes, but not always, clearly due to air cysts with well defined walls. We did not consider that transient patchy opacities caused by atelectasis, infection, or haemorrhage that were superimposed on this pattern altered the definition of type 2 (fig 3).

The clinical features of infants with the two types of pattern were compared by the Mann-Whitney U test, which was used to obtain estimates and 95% confidence intervals (CI) for differences between the medians in the two groups; a confidence interval that includes zero indicates that the difference is not significant at the 5% level.

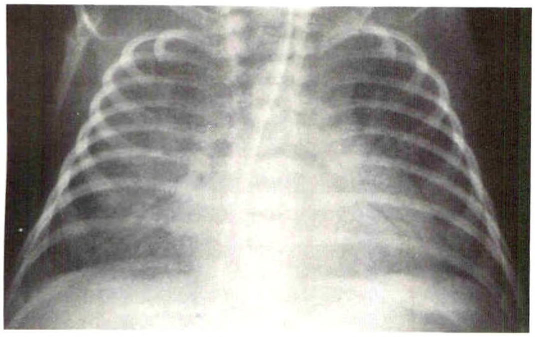

Fig 1 *Type 1 chronic lung disease in an infant aged 37 days. Note bilateral hazy perihilar opacities with no coarse reticulation.*

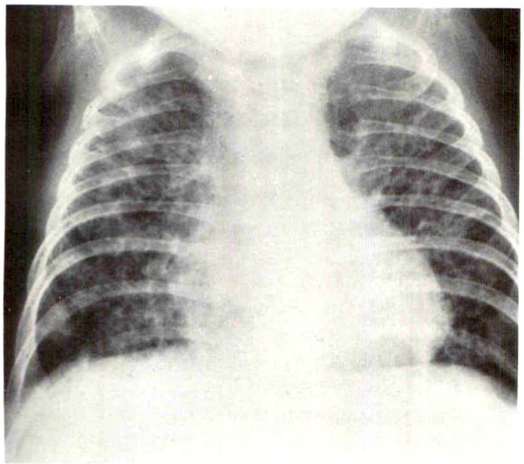

Fig 2 *Type 2 chronic lung disease in an infant aged 88 days. Note generalised coarse reticulation.*

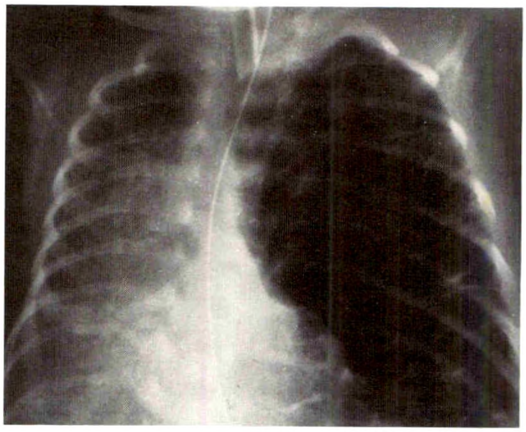

Fig 3 *Type 2 chronic lung disease in an infant aged 23 days. Note transient homogeneous opacity of the right lung due to compression collapse from the contralateral air cysts.*

Results

Thirty four infants were included in the study, of whom 33 met all the criteria described. The exception was an infant who died at 21 days of age and in whom bronchopulmonary dysplasia was found at necropsy. Thirty one were ventilated for hyaline membrane disease; three had normal chest radiographs at birth and were presumably ventilated because of apnoea.

There were 19 patients with type 1 and 11 with type 2 disease. Four infants could not be classified; of these, three started as type 1 but became type 2, and in one a type 2 pattern became type 1.

Table 1 shows the birth weights and gestation periods of the infants for each type. The median birth weight for type 2 infants was estimated to be 100 g higher than for type 1 (95% CI of the

Table 1 *Details of patients*

Median range	*Type 1 chronic lung disease of prematurity (n=19)*	*Type 2 chronic lung disease of prematurity (n=11)*
Body weight (g)	980 (660–1520)	1080 (800–1600)
Gestation (weeks)	27 (25–30)	28 (25–31)

Table 2 *Complications*

	Type 1 chronic lung disease of prematurity *No (%)*	*Type 2 chronic lung disease of prematurity* *No (%)*
Pulmonary interstitial emphysema	7 (37)	11 (100)
Pneumothorax	5 (26)	9 (82)
Patent ductus arteriosus:		
Total	13 (68)	8 (73)
No requiring ligation (% of total)	5 (26)	4 (36)
Total	19 (100)	11 (100)

Table 3 *Details of ventilation regimens*

	Type 1 chronic lung disease of prematurity	*Type 2 chronic lung disease of prematurity*
Median (range) period of ventilation (days)	30 (6–99)	40 (21–109)
Median (range) period of oxygen dependence (days)	54 (32–113)	48 (21–169)
Median (range) maximum % inspired oxygen	80 (35–100)	100 (25–100)
Minimum and maximum ventilation pressures (cm H_2O)	16/2 to 36/5	20/3 to 38/6

difference −90 to 360 g). For gestation periods the difference was one day (95% CI −1 to 2). All patients with the type 2 pattern had had an episode of pulmonary interstitial emphysema, usually during the first few days of life (table 2). Pulmonary interstitial emphysema usually developed gradually into bronchopulmonary dysplasia, but this was not invariably the case. Patency of the ductus arteriosus was diagnosed in a large proportion of both types and ligation was carried out in about half (table 2).

Both groups required prolonged ventilation (table 3); the median for type 2 was estimated to be 10 days longer than for type 1 (95% CI −5 to 23 days). By definition both groups were dependent on oxygen for some time, the median period being six days longer for type 1 than for type 2 (95% CI −31 to 16 days). Type 2 patients required a significantly higher median maximum percentage concentration of inspired oxygen (p=0·03); the estimated difference in medians was 15% (95% CI 0 to 30%).

There were four deaths, all of type 2 disease. The age at death ranged from 21–169 days.

Discussion

When bronchopulmonary dysplasia was first described it was attributed to the adverse effects of oxygen on the immature lungs of premature infants who were being kept alive by mechanical ventilation.[2] Four stages were identified: (1) two to three days, with the classical radiographic picture of hyaline membrane disease; (2) four to 10 days with almost total opacification of the lungs; (3) 10–20 days, during which the transition to chronic lung disease took place and the chest radiograph showed small rounded translucent areas alternating with areas of irregular density; and (4) after one month, when there was enlargement of the rounded translucencies alternating with thinner strands of density.

It soon became apparent that there were difficulties in staging by radiographic pattern; in particular stage 2 occurred rarely and stage 4 was often atypical.[5] This lead to other criteria being used to define the disease based more on functional and clinical features. Bancalari *et al* required a minimum of three days of mechanical ventilation and 28 days dependence on oxygen, but still included a radiographic pattern of strands of density.[7] Tooley, on the other hand, accepted among the criteria any pulmonary parenchymal abnormality on the chest radiograph at 1 month.[6]

A consensus has emerged that bronchopulmonary dysplasia or chronic lung disease of prematurity can be said to exist in an infant who has required mechanical ventilation for a variable period soon after birth, who remains dependent on supplemental oxygen at 28 days, and still has evidence of pulmonary insufficiency. The arbitrary period of 28 days has the advantage that it usually excludes other conditions with an abnormal chest radiograph that might be confused with bronchopulmonary dysplasia—for example, resolving hyaline membrane disease, patent ductus arteriosus with heart failure, pulmonary haemorrhage, and infection.

The justification for the assumption that both the radiological patterns that we have described are caused by bronchopulmonary dysplasia is that they fulfill the accepted criteria of predisposing cause, chronic pulmonary disease, and long term dependence on supplemental oxygen. The occasional transition from one type to the other strengthens that assumption.

The respiratory insufficiency syndrome,[8] chronic pulmonary insufficiency of prematurity,[9] and Wilson-Mikity syndrome (pulmonary dysmaturity),[10] are diseases that characteristically develop after an initial period free of respiratory problems. Of these, only the Wilson-Mikity syndrome has any radiographic similarity to bronchopulmonary dysplasia. Edwards *et al* reported a group of premature infants with lung disease that they called the 'immature lung',[11] with radiographic appearances corresponding closely to what we would call type 2 bronchopulmonary dysplasia; the main difference was the length of time on supplemental oxygen, a factor that was built into our case selection. They believed that these infants had not had respiratory distress syndrome partly because the biochemical profile of pulmonary surfactant was normal.

In the theories of the aetiology of bronchopulmonary dysplasia the role of oxygen toxicity has been downgraded and that of mechanical ventilation promoted, to the extent that the term 'barotrauma' is now sometimes used. Type 1 bronchopulmonary dysplasia as defined by Henghan *et al*,[4] and in the present series, is less serious than type 2, having no mortality and less morbidity, and a lower incidence of air leaks. These are also features of the immature lung. In the absence of histological confirmation the diagnosis of bronchopulmonary dysplasia is imprecise and it is probable that there is overlap with immature lung, respiratory insufficiency syndrome, chronic pulmonary insufficiency of prematurity, and the Wilson-Mikity syndrome.

Pulmonary interstitial emphysema arising in the course of respiratory distress syndrome is a bad prognostic sign and is associated with a higher incidence of bronchopulmonary dysplasia.[12] In the present series all the cases that developed the classical radiographic pattern of bronchopulmonary dysplasia—that is type 2 disease—had this complication. Its appearance is sudden and dramatic and its resolution is usually also rapid, but a persistent form has been described,[13] which (if bilateral and generalised) is readily confused with bronchopulmonary dysplasia stages 3 and 4 as described by Northway *et al*.[2]

If it is accepted that the homogeneous opacity with a predominantly perihilar distribution truly reflects bronchopulmonary dysplasia then it follows that there are two distinct radiographic patterns of the disease. This study has provided no evidence of a significant difference between the two types with respect to birth weight or period of gestation. We suggest that type 1 represents the basic or pure form, and that the coarse reticular type 2 is a complicated form usually attributable to pulmonary interstitial emphysema.

The pathological basis of the type 1 change is unknown. Alveolar oedema usually shows short term changes in intensity and distribution either spontaneously or as a result of treatment with diuretics. Pulmonary fibrosis should be permanent whereas the type 1 change eventually resolves. For these reasons neither pulmonary oedema nor fibrosis are likely causes; a cellular infiltration seems the most likely explanation. It may be relevant that in the series of immature lung four of those who died had thickened hypercellular alveolar walls, and one also had a chronic interstitial infiltrate that suggested bronchopulmonary dysplasia.[11]

References

[1] Avery ME, Fletcher BD, Williams RG. *The lung and its disorders in the newborn infant.* 4th ed. Philadelphia: WB Saunders, 1981:225.

[2] Northway WH, Rosan RC, Porter DY. Pulmonary disease following respirator therapy of hyaline-membrane disease. *N Engl J Med* 1967;**276:**357–68.

[3] Cleveland RH, Todres ID. Patterns of evolution of x-ray changes in respiratory distress syndrome. *Helv Paediatr Acta* 1981;**36:**43–53.

[4] Heneghan MA, Sosulski R, Baquero JM. Persistent pulmonary abnormalities in newborns: the changing picture of bronchopulmonary dysplasia. *Pediatr Radiol* 1986;**16:**180–4.

[5] Edwards DK. Radiographic aspects of bronchopulmonary dysplasia. *J Pediatr* 1979;**95:**823–9.

[6] Tooley WH. Epidemiology of bronchopulmonary dysplasia. *J Pediatr* 1979;**95:**851–5.

[7] Bancalari E, Abdenour GE, Feller R, Gannon J. Bronchopulmonary dysplasia: clinical presentation. *J Pediatr* 1979; **95:** 819–23.

[8] Carlsson J, Svenningsen NW. Respiratory insufficiency syndrome (RIS) in preterm infants with gestational age of 32 weeks and less. *Acta Paediatr Scand* 1975;**64:**813–21.

[9] Krauss AN, Klain DB, Auld PAM. Chronic pulmonary insufficiency of prematurity (CPIP). *Pediatrics* 1975;**55:**55–8.

[10] Wilson MG, Mikity VG. A new form of respiratory disease in premature infants. *AMA Journal of Diseases of Children* 1960;**99:**489–99.

[11] Edwards DK, Jacob J, Gluck L. The immature lung: radiographic appearance, course and complications. *AJR* 1980;**135:** 659–66.

[12] Moylan FMB, Walker AM, Kramer SS, Todres ID, Shannon DC. Alveolar rupture as an independent predictor of bronchopulmonary dysplasia. *Crit Care Med* 1978;**6:**10–3.

[13] Stocker JT, Madewell JE. Persistent interstitial pulmonary emphysema: another complication of the respiratory distress syndrome. *Pediatrics* 1977;**59:**847–57.

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Continuous EEG monitoring of neonatal seizures: diagnostic and prognostic considerations

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SUMMARY We recruited 275 full term and preterm infants into a prospective evaluation of continuous four channel electroencephalographic (EEG) monitoring in the diagnosis and prognosis of neonatal seizures. EEG seizure activity was found in 55 infants; clinical signs were completely simultaneous in only 12 of these, they were present but limited in another 20, and were completely absent in the remaining 23. EEG seizure activity, with or without clinical signs, were equally associated with serious cerebral lesions and with adverse clinical outcome.

The four channel EEG recording provided sufficient data on abnormality to be prognostically specific in 79% of the 43 infants who either died or had serious neurological impairment.

The technical suitability of the Oxford Medilog continuous four channel electroencephalographic (EEG) recorder for diagnostic monitoring of neonatal seizures was first reported by Eyre *et al* in 1983.[1] Their study of prospective monitoring for the first five days of life in high risk neonates showed that there was a high incidence of EEG seizure activity in infants in whom clinical signs were either completely undetected or had apparently ceased in response to treatment with anticonvulsant drugs. In contrast, Bridges *et al*[2], using the Medilog more selectively in infants in whom standard recording had already failed to show seizures and whose clinical signs had usually already ceased, found a much lower incidence of EEG seizure activity and concluded that continuous EEG monitoring was unlikely to be of diagnostic value except when clinical seizures continued. Neither of these studies commented on the prognostic significance of their observations, although the conventional multichannel EEG recording is well established as a sensitive guide to outcome in neonatal seizures.[3–9]

Despite its technical advantages over conventional recording, extensive use of the Medilog monitor is still expensive and time consuming. There is still a need therefore for further comprehensive evaluation of the diagnostic and prognostic potential of this technique as a guide to its optimal application in neonatal seizure disorders.

The aim of the present study was to undertake such an evaluation within the high risk population of our regional neonatal referral unit with particular attention to: the incidence of EEG seizure activity within this population; the extent to which EEG seizure activity was accompanied by clinical signs; the importance of EEG and clinical seizures in relation to concurrent clinical features and subsequent neurological outcome; the prognostic significance of EEG background activity and of quantitative analysis of seizure frequency and duration; and the establishment of criteria for optimal clinical use of the Medilog in the evaluation of neonatal seizures.

Patients and methods

During the two year period March 1985 to March 1987, 275 patients were recruited to this study from 603 consecutive admissions to our regional neonatal referral unit. Admission to the study was from one of two main sources:

PROSPECTIVE MONITORING (259 INFANTS)

Subject only to the availability of recorders and of personnel to apply them, infants were monitored consecutively according to their degree of clinical risk during two separate phases. Firstly, during an initial six month period, when equal priority was given to recruitment of low risk infants to establish normal values and, secondly, during a subsequent 18 month period when high risk infants were preferentially studied.

High risk infants all had some predisposing feature; in particular, the category included those with congenital malformations of the central nervous system, birth asphyxia, respiratory distress, sepsis, metabolic disturbance, or evidence of intraventricular haemorrhage or leucomalacia on cranial ultrasound scan.

CLINICAL SEIZURES

Sixteen infants with seizures that were diagnosed clinically before prospective monitoring could be started were also studied; 10 had developed seizures in the unit before the monitor was applied, and six were transferred from other hospitals with established seizures.

For the purpose of this study, we defined 'neonatal' seizures as those occurring in infants up to 44 weeks' postmenstrual age, irrespective of their actual gestational age at birth.

Diagnosis of clinical seizures was based entirely on observations by nursing or medical staff. Clinical seizure types were classified according to Volpe as myoclonic, focal clonic, multifocal clonic, general tonic, or subtle.[10] Other acute associations were also based on clinical observations and findings from serial cranial ultrasound scanning performed with an Advanced Technology Laboratories Mark III sector scanner using a 5 or 7·5 MHz transducer.

EEG recordings were performed using the Oxford Medilog 4–24 continuous four channel monitor.[1] One channel was allocated to electrocardiography,[2] two were allocated to the EEG, recorded with silver/silver chloride electrodes applied with collodion in the F4–P4 and F3–P3 positions (impedances <5 kohms) according to the method described by Eyre *et al*,[1] and the fourth channel to either respiration or a time-event marker.

Prospective early recordings were made within the first 72 hours of life in all high risk infants, and in all but three low risk infants who were monitored on the fourth day. Low risk infants were monitored for at least 24 hours and high risk infants for at least 48 hours. In infants with clinical or EEG seizure activity or both, monitoring was continued throughout these events and for at least 24 hours, or until death.

Later monitoring was performed either as routine follow up in stable infants at two to four weekly intervals while they remained within the unit, or if acute deterioration occurred in the infant's condition. In the former, recordings were made for 24 hours, and in the latter, for a minimum of 48 hours and then discontinued if no seizures were found. If seizures were detected recording was continued for at least 24 hours after seizures ceased, or until death.

Each 24 hours of data was recorded on standard C–120 cassette tape and reviewed on the visual display unit of the Medilog system at 20 and 60 times the normal speed at scales of 1·5 and 3·0 cm/s. A compressed transcription for further analysis was made at 6 cm/minute using a Siemens 8 Elema Schonander ink jet recorder.

EEG recordings were analysed in relation to normal standards for gestational age established from previous studies using standard EEG recording,[3 4 11 12] and also from our own study specifically for continuous four channel recording.[13]

EEG seizure activity was identified by the characteristics reported in previous studies[3 14] and was analysed quantitatively for maximum daily frequency (EEG seizure activity/24 hour recording), maximum daily seizure time (number of hours/day occupied by seizures), and total duration of EEG seizure activity (days).

Background EEG was allocated to one of the following categories: normal or abnormal; if the latter they were subdivided into one of the following:

1—Excess amounts of discontinuous activity/24 hours in relation to gestational norms, but no other abnormality. This could be: (a) transient (24 hours only), or (b) sustained (more than 24 hours).

2—Excess discontinuity as in category 1, plus one or more of the following: asynchrony; asymmetry; prolonged interburst interval for gestation, and low amplitude. This was further subdivided into: (a) transient (24 hours only), or (b) sustained (more than 24 hours).

3—Maximal background depression with no discernible electrical activity for more than one hour: (a) as culmination of a sequence of progressive deterioration of activity with steadily increasing interburst intervals and decreasing burst duration and amplitude, or (b) as an established finding at the first recording.

EEG recordings were analysed by two independent observers who were not regularly concerned in the clinical management of the infants studied. Results of EEG monitoring were not made available to clinical staff unless specifically requested and were then communicated without any recommendation regarding decisions on clinical management.

Assessment of outcome was based on clinical neurological examination at discharge from the unit using a standardised protocol,[15] and subsequently at regular intervals between six and 24 months using the Griffiths's mental developmental scale and items from Amiel-Tison and Grenier,[16] and Touwen.[17] On the basis of this, infants were allocated to one of four groups: normal outcome, transient abnormalities of tone but no other abnormality,[18] major neurological abnormality (cerebral palsy or mental retardation or both), or death.

To assess the relative diagnostic contribution of continuous Medilog monitoring, clinical signs of seizures and EEG seizure activity were correlated and allocated to one of the following categories: simultaneous EEG and clinical seizure activity, EEG seizure activity preceding or outlasting clinical events, or both, or EEG seizure activity with no clinical signs. Infants with no detectable EEG seizure activity despite a clinical diagnosis of seizures were assessed separately.

The relative importance of the above combinations of EEG and clinical seizures was assessed by comparing the acute aetiological associations and clinical neurological outcome in the infants concerned.

The prognostic function of the four channel Medilog recording was assessed by relating clinical neurological outcome of the infants studied to their background EEG activity and to quantitative aspects of seizures detected by the two EEG channels.

Results

INCIDENCE OF EEG SEIZURES (TABLE 1)

During this two year study continuous EEG monitoring was carried out in 275 infants, 45·6% of the total 603 admitted to our unit. During the initial six months, when both high and low risk infants were equally eligible, 64·8% of all admissions were monitored. In the following 18 months when mainly high risk infants were studied, monitoring was carried out in 38·2% of all infants admitted.

The incidence of EEG seizure activity was consistent in both the subdivided and total study periods. They were found in 20% of all infants admitted and in 25% of high risk cases in both periods. EEG seizure activity was never seen in low risk infants.

RELATION OF CLINICAL AND EEG SEIZURE ACTIVITY

Of the 55 infants with EEG seizure activity, 23 (42%) had no clinical signs, nine of them being paralysed for ventilation. There were a further 20 (36%), in whom EEG seizure activity either preceded (by a mean of 16 hours) or outlasted (by a mean of 32 hours) the clinical signs; six of these were therapeutically paralysed. Simultaneous EEG and clinical seizures were found in only 12 infants.

Seven infants had clinically diagnosed seizures that were not detected by EEG. They showed no important differences in associated clinical features or outcome from those with EEG seizure activity and are discussed separately.

COMPARATIVE ASSOCIATIONS OF CLINICAL AND EEG SEIZURE ACTIVITY

Postmenstrual age at onset (table 2)

Postmenstrual age (gestational age plus chronological age) at onset of seizures was less than 32 weeks in 22, between 32 and 37 weeks in 14, and more than 37 weeks in 19 infants.

The diagnostic yield from EEG monitoring was greatest in infants of less than 32 weeks' postmenstrual age. Of the unparalysed infants of this age, 70% had no clinical signs of EEG seizure activity. In addition, this group contained 12 out of 15 of all infants in this study who had therapeutic paralysis.

With increasing maturity, simultaneous EEG and clinical seizures became more frequent but were still found in only 47% of unparalysed infants of more than 37 weeks' postmenstrual age.

Clinical seizure types (table 3)

Thirty two of the 55 infants with EEG seizure activity had clinical signs. There were no significant differences in the types of clinical seizures among

Table 1 *Incidence of seizures among neonates and clinical risk during the study periods*

	Study period		*Total*
	0–6 months	*7–24 months*	
Total No of seizures	20	35	55
No of high risk infants	75	145	220
No (%) with seizures	20 (27)	35 (24)	55
No of low risk infants	34	21	55
No (%) with seizures	0	0	0
Total No of infants monitored	109	166	275
No (%) with seizures	20 (18)	35 (21)	55 (20)
Total No of admissions	168	435	603
No (%) with seizures	20 (12)	35 (8)	55 (9)

Expressing the number of seizures as a percentage of the total number of admissions permits estimate of the minimum incidence of seizures during these periods.

Table 2 *Extent of clinical signs of EEG seizure activity and total gestational age in 55 infants*

	Postmenstrual age (weeks)			*Total*
	<32	*32–37*	*>37*	
Total No of clinical seizures	22	14	19	55
No with simultaneous clinical signs	1	3	8	12
No with limited clinical signs				20
Therapeutically paralysed	5	0	1	6
Not paralysed	2	5	7	14
No with absent clinical signs				23
Therapeutically paralysed	7	1	1	9
Not paralysed	7	5	2	14

Table 3 *Extent of clinical signs of seizures and Volpe classification in 32 infants**

Type of seizure	*Clinical signs*		*Total*
	Simultaneous	*Limited*	
Subtle	6	8	14
General tonic	6	10	16
Multifocal clonic	8	9	17
Focal clonic	1	1	2
Myoclonic	0	1	1

*17 infants had more than one type of seizure.

the 12 infants whose clinical manifestations occurred simultaneously and the 20 in whom they were limited. These 32 infants each showed between one and three seizure types. The types most commonly detected were multifocal clonic (34%), general tonic (32%), and subtle (28%). Seventeen infants showed more than one seizure type. In the other 15, subtle clinical seizure activity was the sole sign in only two cases.

Aetiological associations associated with postmenstrual age

Forty seven of the 55 infants with EEG seizure activity had one of three main aetiological associations at onset associated with postmenstrual age (table 4). Intraventricular haemorrhage was the commonest association at less than 32 weeks', leucomalacia between 32 and 37 weeks', and hypoxic-ischaemic encephalopathy at more than 37 weeks' postmenstrual age. In the other eight infants the main associations were transient flares on ultrasound imaging in three cases (all of less than 32 weeks' postmenstrual age), metabolic abnormalities in two infants of more than 37 weeks (1 had hyponatraemia, the other a urea cycle defect), and sepsis in three infants of varying ages.

Table 4 *Principal aetiological associations and total gestational age in 55 infants with neonatal EEG seizure activity*

Aetiological associations	*Postmenstrual age (weeks)*			*Total (n=55)*
	<32 (n=22)	*32–37 (n=14)*	*>37 (n=19)*	
Main associations:				
Hypoxic-ischaemic encephalopathy	1	4	14	19
Leucomalacia	4	6	1	11
Periventricular haemorrhage	13	3	1	17
Other associations:				
Metabolic	0	0	2	2
Flares	3	0	0	3
Sepsis	1	1	1	3

Severe cerebral lesions were present equally in those with or without clinical signs. Because the detectability of clinical signs was related to the maturity of the infant, however, intraventricular haemorrhage (the predominant lesion in those of less than 32 weeks' gestation) was also most commonly associated with EEG seizure activity without clinical expression. Conversely, simultaneous EEG and clinical seizures were most commonly found in hypoxic-ischaemic encephalopathy, which was the main aetiological association in infants of more than 37 weeks' postmenstrual age. Details of the association of seizure aetiology and degree of clinical expression of EEG seizure activity are given in table 5.

PROGNOSTIC ASSOCIATIONS

Outcome was generally extremely poor in the 55 infants with EEG seizure activity. Thirty infants died and a further 13 survived with major neurological abnormality. Transient signs of dystonia were present in eight, and only four had a completely normal neurological outcome. Later epilepsy has so far been seen in only one infant who also has a major neurological abnormality.

Outcome in clinical and EEG seizure activity (table 6)

There was no significant difference in outcome between infants with EEG seizures only and those with simultaneous or limited clinical signs.

Prognostic importance of EEG analysis

Background EEG activity (table 7)—Normal background EEG activity between seizures was usually,

Table 5 *Extent of clinical signs of EEG seizure activity and aetiological association in 55 infants*

Clinical seizures	*Aetiological association*				*Total*
	Hypoxic-ischaemic encephalopathy	*Leucomalacia*	*Periventricular haemorrhage*	*Other*	
Simultaneous	6	3	0	3	12
Limited	9	3	7	1	20
Absent	4	5	10	4	23

Table 6 *Neurological outcome and extent of clinical signs of EEG seizure activity in 55 infants*

Clinical seizures	*Neurological outcome*				*Total*
	Normal (n=4)	*Dystonia (n=8)*	*Major abnormality (n=13)*	*Death (n=30)*	
Simultaneous	1	1	2	8	12
Limited	2	1	5	12	20
Absent	1	6	6	10	23

Table 7 *Background EEG activity and neurological outcome in 55 infants with neonatal EEG seizure activity*

Background EEG activity	*Neurological outcome*				*Total*
	Normal (n=4)	*Dystonia (n=8)*	*Major abnormality (n=13)*	*Dead (n=30)*	
Normal	3	3	2	2	10
Abnormal					
1 Excess discontinuity:					
(a) Transient	0	3	0	2	5
(b) Persistent	0	2	2	6	10
2 Excess discontinuity with other abnormality:					
(a) Transient	1	0	0	2	3
(b) Persistent	0	0	7	1	8
3 Maximal depression:					
(a) Gradual onset	0	0	0	14	14
(b) Present throughout	0	0	2	3	5

but not invariably, associated with normal neurological outcome. Excessive discontinuous EEG activity when found as an isolated abnormality was associated mainly with transient dystonia in surviving infants. Outcome became worse with increasing severity of background EEG abnormality. All those with persistently asynchronous, asymmetrical, or low amplitude traces either died or had major neurological abnormality if they survived. None of these infants had plasma phenobarbitone concentrations above the therapeutic range.

Quantitative features of EEG seizure activity—All 22 infants who exceeded one or more of the following quantitative measurements of frequency and duration of EEG seizure activity either died or had major neurological abnormality if they survived: a peak frequency of more than 25 seizures/day (n=17); total daily duration of seizure activity more than 30 minutes (n=18); or persistence of seizure activity for more than 48 hours (n=13). Less frequent or prolonged EEG seizure activity, however, as found in the other 33 infants did not necessarily guarantee survival without major neurological abnormality.

Relative prognostic contribution of background and quantitative analysis—Twenty two infants had one or more of the quantitative seizure abnormalities consistently associated with major neurological abnormality or death. This was additionally predictive of major abnormality or death in one infant with normal background EEG, and confirmatory in another five with background EEG abnormalities of intermediate severity. In the remaining 16 infants, quantitative analysis did not add to the prediction of major abnormality or death already made from the presence of severe background EEG abnormality.

Thirty three infants had EEG seizure activity of insufficient frequency and duration to allow definite prediction of adverse outcome, but this was further established in 12 of these by analysis of the background EEG, which showed activity constantly associated with major abnormality or death.

Thus the combined background and quantitative analysis of the four channel Medilog recording permitted certain prediction of seriously adverse outcome in 34 of the 43 infants who died, or survived with major neurological abnormality.

Discussion

Our findings confirm those of Eyre *et. al*[1] that neonatal seizures are more frequently diagnosed by continuous EEG monitoring than by clinical observation. In our extensive study of 275 neonates recruited during a continuous two year period, we detected an overall minimum incidence of EEG seizure activity of 20% in all infants monitored, which rose to 25% in high risk cases. It seems likely that if it had been possible to study more high risk infants for longer periods, this overall incidence would have been even higher.

The relative diagnostic contribution of the Medilog is emphasised by the limitation or absence of clinical signs in 78% of infants with EEG seizure activity. The study of Eyre *et al*, though reporting a much higher incidence of EEG seizure activity in a smaller study group (20 of 25), found a remarkably similar proportion to be without clinical signs: nine of 20 (45%) compared with our figure of 23 of 55 (42%). This suggests that although the incidence of neonatal EEG seizure activity may vary with the population studied, their limited association with clinical signs is a more constant feature.

The seven infants who had clinical seizures without EEG signs are of particular interest. Clinical diagnosis may have been incorrect, but this should not be assumed and it is important to consider why the Medilog may have failed to detect EEG seizure activity in these seven cases. The recordings may have been carried out at inappropriate times, which may explain three cases in which clinical events had ceased before application of the monitor. In four others, however, recording was carried out during clinical events. Severe amplitude depression of background EEG activity is well recognised from standard recording as impeding the display of electrical seizure activity.[3 6] This was found in two infants. We cannot determine the reason in the remaining two infants, but a further possibility is that the limited number of recording channels may have failed to detect focal seizure activity.

The data provided by these two EEG channels are usually sufficient to fulfil the prognostic function already shown by standard EEG recording.[3–9] This function is reliable even when anticonvulsant drugs are being given within the therapeutic range.[19] Normal background EEG activity was predominantly, though not invariably, associated with normal clinical outcome. Infants with abnormal background activity rarely had a normal outcome. Those with persistently asymmetrical, asynchronous, and low amplitude activity, together with those who showed maximal background depression (either as a presenting feature or as the culmination of a progressive EEG deterioration) all either died or had major neurological abnormalities if they survived.

These important background abnormalities should be equally detectable by intermittent or by continuous monitoring. Continuous recording did, however, permit the additional detection of quantitative EEG features that were also constantly associated with major neurological abnormality or death. These were a maximum seizure frequency of more than 25/day, a maximum daily seizure duration of more than 30 minutes, and a total EEG seizure duration of more than 48 hours. These quantitative factors provided helpful confirmation of prognosis based on background analysis but usually did not add to it.

Diagnosis of neonatal seizures has been regarded as important in view of their close association with severe cerebral lesions and the extremely poor outcome of infants in whom they occur.[3 10 20] It is not possible from this study to determine the relative contributions of the underlying brain lesion and of the seizures themselves to subsequent neurological abnormality, nor can we determine whether clinical and non-clinical seizures act through identical pathophysiological mechanisms. We did, however, find both to be equally associated with severe brain lesions and adverse neurological outcome. We suggest that equal concern should be aroused by non-clinical (EEG) as by clinical seizures, and that their detection be regarded as equally important.

In the detection of non-clinical seizures, optimal diagnostic return from limited resources may be achieved by restricting prospective monitoring to the highest risk period for seizures, which is usually in the first 48 to 72 hours after birth, or after acute collapse at a later stage. Priority should be given to infants with the highest potential incidence of non-clinical seizures, which in our study were those of less than 32 weeks' postmenstrual age, and neonates of any age paralysed for ventilation in whom seizures would not be apparent. Infants in whom clinical seizures have been diagnosed in the previous 24 to 48 hours may still gainfully be monitored in view of the persistence of EEG seizure activity for a mean of 32 hours after clinical events had apparently ceased.

During this diagnostic period of continuous monitoring, assessment may also be made for prognostic purposes of important quantitative aspects of EEG seizure activity. Prognostic features of EEG background activity may also be detected during this time. In infants with significant background abnormalities persisting after seizures have ceased, intermittent recordings of 1 to 2 hours/day at intervals determined by rate of resolution would be adequate for prognostic purposes.

In conclusion, we suggest that the diagnostic and prognostic value of the four channel Medilog monitor, combined with its practical advantages for use in neonatal intensive care, should make this the technique of choice for EEG evaluation of neonatal seizure disorders.

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References

[1] Eyre JA, Oozeer RC, Wilkinson AR. Diagnosis of neonatal seizure by continuous recording and rapid analysis of the electroencephalogram. *Arch Dis Child* 1983;**58**:785–90.

[2] Bridges SL, Ebersole JS, Ment LR, Ehrenkranz RA, Silva CG. Cassette electroencephalography in the evaluation of neonatal seizures. *Arch Neurol* 1986;**43**:49–51.

[3] Werner SS, Stockard JE, Bickford RG. *Atlas of neonatal electroencephalography.* New York: Raven Press, 1977.

[4] Dreyfus-Brisac C. Neonatal electroencephalography. *Reviews in Perinatal Medicine* 1979;**3**:397–471.

[5] Engel R. *Abnormal electroencephalogram in the neonatal period.* Springfield: Thomas, 1975.

[6] Monod F, Dreyfus-Brisac C, Sfaello Z. Depistage et prognostic de l'etat de mal neo-natal d'apres l'etude de 150 cas. *Arch Fr Pediatr* 1969;**26**:1085–102.

[7] Rose AL, Lombroso CT. A study of clinical, pathological and electroencephalographical features in 137 full-term babies with a long-term follow up. *Pediatrics* 1970;**45**:404–25.

[8] Rowe JC, Holmes GL, Hafford J. Prognostic value of the electroencephalogram in term and preterm infants following neonatal seizures. *Electroencephalog Clin Neurophysiol* 1985; **60**:183–96.

[9] Tharp BR, Cukier F, Monod N. The prognostic value of the electroencephalogram in preterm infants. *Electroencephalog Clin Neurophysiol* 1981;**51**:219–36.

[10] Volpe JJ. Neonatal seizures. *Neurology of the newborn.* Philadelphia: Saunders, 1987:129–59.

[11] Anderson CM, Torres F, Faoro A. The EEG of the early premature. *Electroencephalog Clin Neurophysiol* 1985;**60**: 95–105.

[12] Lombroso CT. Neonatal polygraphy in full-term and preterm infants: a review of normal and abnormal findings. *J Clin Neurophysiol* 1985;**2**:105–55.

[13] Connell JA, Oozeer R, Dubowitz V. Continuous 4-channel EEG monitoring: a guide to interpretation, with normal values, in preterm infants. *Neuropediatrics* 1987;**18**:138–45.

[14] Harris R, Tizard JPM. The electroencephalogram in neonatal convulsions. *J Pediatr* 1960;**57**:501–21.

[15] Dubowitz LMS, Dubowitz V. *The neurological assessment of the preterm and full-term infant. Clinics in developmental medicine, No 79.* London: Heinemann, 1981. (Spastics International Medical Publications.)

[16] Amiel-Tison C, Grenier A. Evaluation neurologique du nouveau-ne et du nourisson. Paris: Masson: 1980.

[17] Touwen BLC. *Examination of the child with minor neurological dysfunction. Clinics in developmental medicine, No 71.* Oxford: Blackwell, 1979. (Spastics International Medical Publications.)

[18] Drillien CM. Abnormal neurological signs in the first year of life in low-birthweight infants: possible prognostic significance. *Dev Med Child Neurol* 1972;**14**:575–84.

[19] Staudt F, Scholl ML, Coen RW, Bickford RW. Phenobarbital therapy in neonatal seizures and the prognostic value of the EEG. *Neuropediatrics* 1982;**13**:24–33.

[20] Bergman I, Painter MJ, Hirsch RP, Crumrine PK, David R. Outcome in neonates with convulsions treated in an intensive care unit. *Ann Neurol* 1983;**14**:642–7.

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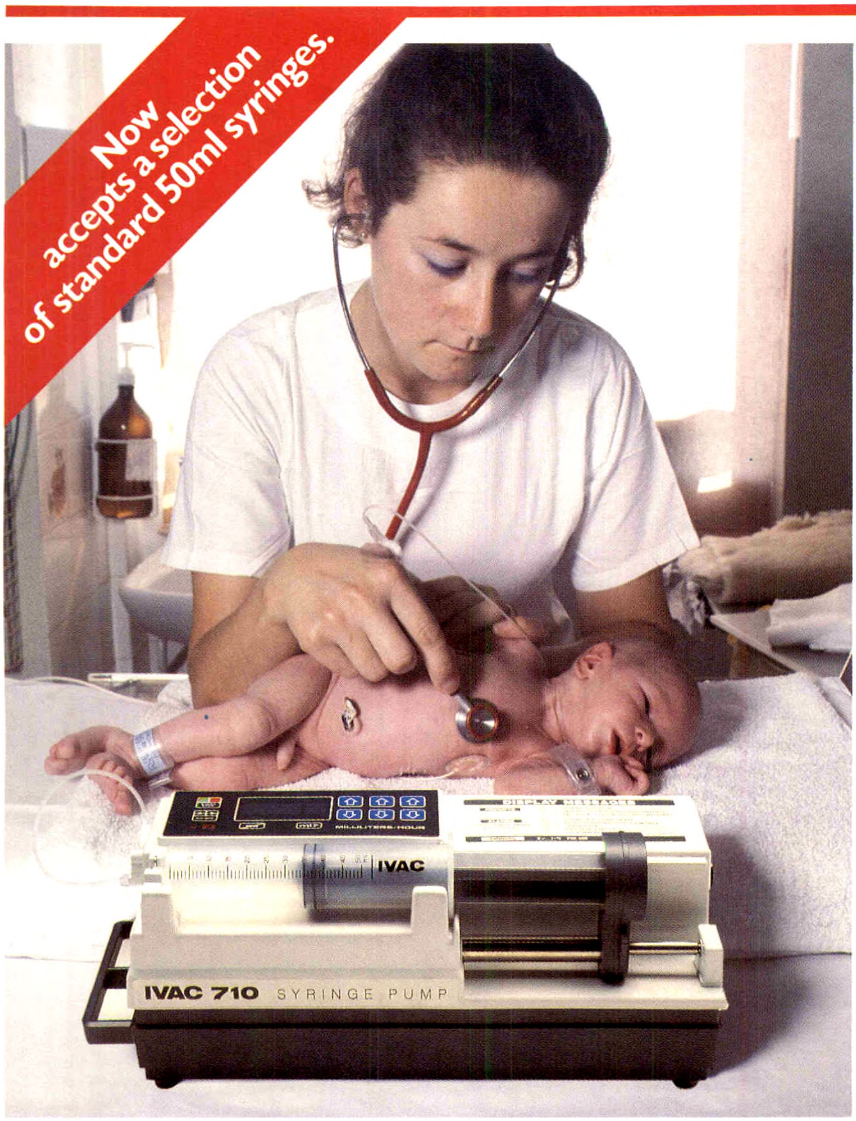

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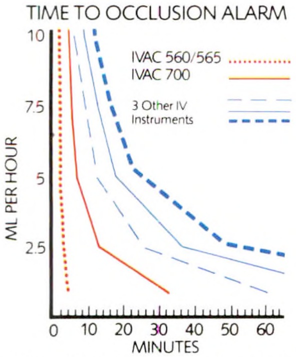

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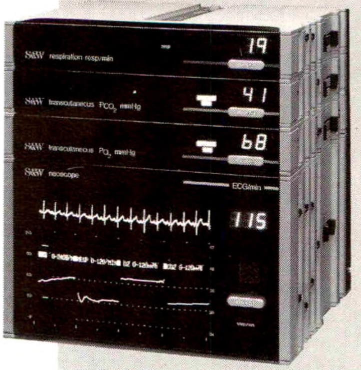

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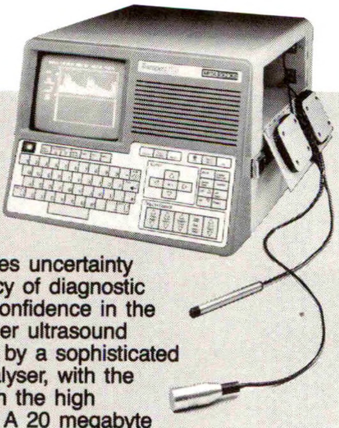

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Clinical and EEG response to anticonvulsants in neonatal seizures

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SUMMARY During a two year period prospective continuous electroencephalographic (EEG) monitoring of 275 infants identified seizure activity in 55 cases, 31 of whom were treated with anticonvulsant drugs on clinical grounds. EEG and clinical response was complete in only two and equivocal in another six. Clinical response with persistent EEG seizures occurred in 13 and neither clinical nor EEG response in 10. There was no significant improvement in the generally poor neurological outcome compared with that in 24 infants whose seizures were not treated because of limited or absent clinical manifestations. Background EEG abnormality (as an index of associated cerebral dysfunction) was a guide to potential lack of response to anticonvulsant drugs; it was also predictive of subsequent clinical outcome irrespective of treatment.

This study shows that commonly used anticonvulsant drugs (phenobarbitone, paraldehyde, phenytoin, and diazepam) have little effect on seizure control or neurological outcome in neonatal seizures associated with haemorrhagic, hypoxic, or ischaemic cerebral lesions. In view of the variable clinical appearance of EEG seizure activity, continuous EEG monitoring should be an essential feature of further study of neonatal anticonvulsant treatment.

Continuous electroencephalographic (EEG) monitoring has identified persistent EEG seizure activity in many infants in whom clinical signs are either undetected or may stop in response to treatment.[1 2] This raises the possibility that many such infants may be failing to receive optimal or adequate anticonvulsant treatment.

Before treatment for EEG detected seizures without clinical signs is contemplated, more critical evaluation of present neonatal anticonvulsant treatment is needed, particularly in view of the potential adverse effects.[3–7]

We have investigated this problem by analysing prospectively the effects of anticonvulsant treatment in all neonates treated for convulsions in our regional referral unit during a two year period. We specifically looked at the response of clinical and EEG seizure activity to anticonvulsant treatment and its association with later neurological outcome.

Patients and methods

During a two year period, 275 infants were recruited to a prospective evaluation of continuous EEG recording with the four channel Oxford Medilog monitor for the diagnosis of neonatal seizures.[2] The 55 infants in whom EEG seizure activity was diagnosed form the study group for this report.

During this time clinical seizures were treated initially with a single dose of one of several anticonvulsant drugs at the discretion of the clinician in the following sequence and dosage until response occurred: phenobarbitone (20 mg/kg intravenously); paraldehyde (0·3 ml/kg per rectum, or 1–3 ml/kg/hour of a 5% intravenous infusion in 5% dextrose); phenytoin (20 mg/kg intravenously); or diazepam (0·25 mg/kg intravenously). Plasma phenobarbitone concentrations were only measured for clinical reasons.

Decisions about the initiation, timing, and continuation of treatment were made independently by the neonatologists who were not given any results of EEG monitoring unless they specifically requested information about a particular child.

EEG recordings were made using the Oxford Medilog 4–24 continuous four channel monitor, according to the method described by Eyre *et al.*[1] Two channels were allocated to EEG, using silver/silver chloride electrodes applied with collodion in the F4–P4 and F3–P3 positions (impedances <5 kohms), one channel to electrocardiography and the fourth channel to either monitoring of respiration, or use as a time-event marker.

Each 24 hours of data was recorded on standard C–120 cassette tape and reviewed on the visual display unit of the Medilog system at 20 and 60 times the normal speed at scales of 1·5 and 3·0 cm/s. A compressed transcription for further analysis was made at 6 cm/minute, using a Siemens 8 Elema-Schonander ink jet recorder.

EEGs were analysed in relation to normal standards for gestational age established from previous studies using standard EEG recording[8–12] and also from our own study specifically for continuous four channel recording.[13]

Background EEG activity was categorised as either normal or abnormal; if the latter, they were allocated to one of the following categories:

1—Percentage of discontinuous activity/24 hours more than two SD above the gestational mean, but no other abnormality; this could be (a) transient (up to 24 hours only), or (b) sustained (more than 24 hours).

2—Excess discontinuity as in category 1, plus one or more of the following: asynchrony, asymmetry, prolonged interburst interval duration (more than two SD above the gestational mean), low amplitude; this could be (a) transient (up to 24 hours only), or (b) sustained (more than 24 hours).

3—Maximal background depression with no discernible electrical activity for more than one hour; (a) as the culmination of a sequence of progressive deterioration of activity with steadily increasing interburst intervals and decreasing burst duration and amplitude, or (b) as an established finding at the first recording.

The efficacy of anticonvulsant drugs in suppressing both clinical and EEG seizure activity was assessed by allocating the response of treated infants into one of the following groups:

Complete response: immediate and sustained cessation of EEG and clinical seizures;

Equivocal response: delayed response (within six hours); poorly sustained response (recurrence within 24 hours);

Persistence of EEG seizure activity after cessation of clinical signs;

No EEG or clinical response. These responses were also evaluated in relation to the postmenstrual age, main aetiological associations, and to the background EEG activity of the infants.

The prognostic importance of these responses was assessed by comparing the clinical neurological outcome in infants in each of the above groups. This assessment was based on examination at discharge from the unit using a standardised form,[14] and at intervals between six and 24 months using the Griffiths's mental development scales[15] and items from Amiel-Tison and Grenier,[16] and Touwen.[17] Infants were then allocated to one of four groups: normal outcome, transient changes in tone but no other abnormality,[18] major neurological abnormality (cerebral palsy or mental retardation, or both), or death.

Comparison was made between the main clinical associations and later outcome of the treated and untreated infants. This was particularly related to background EEG abnormality, which (as a sensitive index of cerebral dysfunction) may permit closer comparison of infants with equivalent degrees of neurological compromise. For this purpose background activity was classed as normal or abnormal. Abnormal background activity was further subclassified as moderately abnormal (categories 1a, 1b, and 2a) or showing major abnormality (categories 2b, 3a, and 3b).

Results

Of the 55 infants with EEG seizures, 31 were treated with anticonvulsant drugs; 30 of these had clinical signs. Twenty four were not treated, only two of whom had clinical signs.

RESPONSE OF EEG AND CLINICAL SEIZURES TO ANTICONVULSANT DRUGS

Of the 31 infants treated, a complete response was found in only two, and another six had an equivocal response. Thirteen had cessation of clinical signs but persistent EEG seizures, and the remaining 10 showed neither EEG nor clinical response.

Responses of individual anticonvulsants (table 1)
Phenobarbitone was used as the first drug of choice in all 31 infants, but achieved complete EEG and clinical response in only two. Equivocal EEG and clinical responses were found in another two infants, and a further six showed apparent clinical response with persistent EEG seizures. The commonest finding, however, was a complete lack of EEG or clinical response and this occurred in the remaining 21 infants treated with phenobarbitone. Only one of these infants had a plasma phenobarbitone concentration below the therapeutic range.

No subsequent anticonvulsant drug produced complete EEG and clinical response. Paraldehyde was used in 15 infants, four of whom had equivocal EEG and clinical responses, two showed clinical response with EEG persistence, and nine had neither EEG nor clinical response. Phenytoin was given to six infants of whom one showed a clinical response with EEG persistence, and the other five had neither EEG nor clinical response. Diazepam was given to seven infants, of whom four showed a clinical response with EEG persistence; the remaining three showed neither EEG nor clinical response. Pyridoxine was also given without EEG or clinical effect to all infants with persistent clinical seizures.

Anticonvulsant response and background EEG activity (table 2)
Of the eight infants with complete or equivocal responses, five had background EEG activity that was either normal or showed only excessive discontinuity as an isolated abnormality. In contrast, of the 23 infants with unresponsive EEG seizures with or without clinical response, only four had the above features while the remaining 19 had more severe abnormalities.

Association of anticonvulsant response and clinical neurological outcome (table 3)
Both infants who responded completely to anticonvulsant drugs had no subsequent persistent abnormality. Of 19 infants who showed equivocal responses or persistent EEG seizures after clinical response, two had a completely normal outcome and one had only transient dystonia, but three had

Table 1 *Responses of 31 infants to specific anticonvulsant drugs (59 doses)*

Response	*Anticonvulsant drug*				*Total No*
	Phenobarbitone (n=31)	*Paraldehyde (n=15)*	*Phenytoin (n=6)*	*Diazepam (n=7)*	
Complete EEG and clinical	2	0	0	0	2
Equivocal	2	4	0	0	6
No clinical, persistent EEG	6	2	1	4	13
No response	21	9	5	3	38

Table 2 *Response to anticonvulsant drugs in 31 patients with background EEG activity*

Response	*Background EEG activity*							*Total No*
	Normal	*Abnormal*						
		1		*2*		*3*		
		a	*b*	*a*	*b*	*a*	*b*	
Complete EEG plus clinical	0	2	0	0	0	0	0	2
Equivocal EEG plus clinical	2	0	1	0	2	1	0	6
No clinical, persistent EEG	0	0	2	2	2	6	1	13
No response	0	0	2	0	0	5	3	10

Table 3 *Response to anticonvulsant treatment and clinical response in 31 patients*

Response	*Clinical neurological outcome*				*Total No*
	Normal (n=2)	*Dystonia (n=3)*	*Major abnormality (n=6)*	*Death (n=20)*	
Complete EEG and clinical	0	2	0	0	2
Equivocal	1	0	1	4	6
No clinical, persistent EEG	1	1	2	9	13
No response	0	0	3	7	10

major neurological abnormalities and the remaining 13 died. Of 10 infants who showed no EEG or clinical responses, seven died, and the three survivors all had major neurological abnormalities.

COMPARISON OF TREATED AND UNTREATED INFANTS (TABLE 4)

The 31 treated and 24 untreated infants differed in several respects. The treated group, with the exception of one infant, had clinical signs and EEG seizures. The exception was a baby boy who was paralysed for ventilation and was treated after a specific request for EEG data from his clinician. In the untreated group only two infants had clinical seizures, and these were not treated because they rapidly resolved. The treated group were also generally more mature (table 4).

Clinical neurological outcome did not differ significantly between the treated and untreated group. The incidence of normal outcome and major abnormality in survivors was similar (table 5). There were more infants with transient dystonia in the untreated group, which also had a lower mortality.

In an attempt to compare the two groups further, infants were subdivided by background EEG appearances so that those with roughly equivalent degrees of cerebral functional disturbance could be compared. All 27 infants with severe background EEG abnormalities (categories 2b and 3), of whom 20 were treated and seven were not untreated, either died or survived with major neurological abnormalities. Nineteen had lesser degrees of background abnormality, but there was no significant difference in outcome between the eight infants who were treated and the 11 who were not.

Outcome in the nine infants who had normal

Table 4 *Comparison of 31 infants treated with anticonvulsant drugs and 24 not so treated*

	Treated with anticonvulsant drugs (n=31)	*Not treated with anticonvulsant drugs (n=24)*	*Total No*
Clinical seizures:			
Present	30	2	32
Absent	1	22	23
Postmenstrual age (weeks):			
<32	8	14	22
32–37	9	5	14
>37	14	5	19
Neurological outcome:			
Normal	2	2	4
Dystonia	3	5	8
Major abnormality	6	7	13
Death	20	10	30

Table 5 *Correlation of clinical neurological outcome and presence of background EEG activity in 55 infants*

Background EEG activity	*Clinical neurological outcome*				*Total No*
	Normal (n=4)	*Dystonia (n=8)*	*Major abnormality (n=13)*	*Dead (n=30)*	
Normal:					
Treated	1	—	—	1	2
Untreated	2	3	2	—	7
Abnormal: moderate (Types 1a, 1b, 2a)					
Treated	1	3	1	4	9
Untreated	—	2	1	7	10
Abnormal: major (Types 2b, 3a, 3b)					
Treated	—	—	5	15	20
Untreated	—	—	4	3	7

background EEG activity was more difficult to compare as only two of these were treated, one of whom died and the other who was abnormal. Of the seven untreated infants, two had a normal outcome, three showed only transient dystonia, and the other two survived with major neurological abnormalities.

Discussion

Although widely used in clinical practice, the efficacy of anticonvulsant drugs in the newborn has never been assessed by controlled trials.[19] In this study we found that the response of neonatal seizures to anticonvulsant drugs was disappointing, and this was especially apparent when the results of continuous EEG monitoring were taken into account. This unexpectedly poor response could be due to population bias in that most of our infants were preterm with major haemorrhagic or ischaemic lesions, and the infants born at full term with hypoxic-ischaemic encephalopathy were largely referrals from other units and thus already preselected for severity.

Poor response to anticonvulsant drugs may be either an intrinsic feature of such lesions in infants of particular states of maturity, or be related to the specific treatment regimen used. The relative importance of these factors, however, is difficult to assess because of the limited understanding of mechanisms determining seizure generation and control in the newborn.[19-22] Previous studies have produced conflicting reports of the benefit of high dose barbiturate treatment to long term outcome.[23 24] Our own findings suggest that phenobarbitone, used as initial treatment in conventional dosage in all infants, was of little or no benefit to most of those treated. None of the other agents were any more effective, but were only used in the more refractory cases and not as initial treatment. A comparative study of alternative sequences of drugs used in the present regimen might determine whether paraldehyde, phenytoin, or diazepam was more effective as drug of first choice. If these proved equally ineffective, alternatives might be sought among anticonvulsant drugs already in established use in older subjects.

Another surprising feature of this study was the absence of any significant difference in neurological outcome in infants with EEG seizures whether treated or not treated with anticonvulsant drugs. Interpretation of this finding must be done with caution because the untreated infants were not recruited and randomised as a formal control group. In particular, they consisted almost entirely of infants without clinical manifestations of EEG seizure activity. Nevertheless, even if different pathophysiological mechanisms cause EEG seizures, with and without clinical manifestations, we have shown elsewhere[2] that they are equally associated with important cerebral lesions. Background EEG activity is an acknowledged index of the degree of cerebral dysfunction,[8-13] even in the presence of commonly used anticonvulsant drugs within the therapeutic range[25 26]; it may thus be of value in comparing treated and untreated infants with equivalent degrees of cerebral dysfunction and help to compensate for imbalances between the groups. In this way we have at least shown that those with extremely abnormal background EEG activity had an equally poor neurological outcome, irrespective of whether they received anticonvulsant drugs, and that those with moderately abnormal background activity derived no clear benefit from treatment.

The effect of anticonvulsant drugs on outcome was more difficult to assess in the nine infants with normal background activity, which is usually associated with normal outcome.[2 8-12] There may be some grounds for concern at the finding of major neurological abnormality in two of seven untreated infants, despite this comparatively favourable prognostic feature. Further study of a larger number of cases may identify a potential advantage to treatment in this group.

On the basis of these findings, the nature and severity of the underlying cerebral lesions seem to be the main determinants of both the frequency and duration of associated seizure activity and the subsequent neurological outcome of the infant. Its implications for neonatal practice suggest that more critical review is needed of both clinical and nonclinical seizures, of the balance between anticipated benefits of the use of anticonvulsant drugs, and their recognised adverse effects, particularly in infants with established cerebral lesions.

Further research may thus optimally be aimed at management of the primary lesion, including assessment of new agents for limiting its progress, such as glutamate antagonists,[27] rather than undertaking exhaustive comparative studies of available anticonvulsant drugs. In any further study, the use of continuous EEG monitoring for accurate diagnosis of seizures and their response to treatment would be essential.

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References

1 Eyre JA, Oozeer RC, Wilkinson AR. Diagnosis of neonatal seizure by continuous recording and rapid analysis of the electroencephalogram. *Arch Dis Child* 1983;**58**:785–90.

[2] Connell J, Oozeer R, de Vries L, Dubowitz LMS, Dubowitz V. Continuous EEG monitoring for neonatal seizures: diagnostic and prognostic considerations. *Arch Dis Child* 1989;**64**:452–8.

[3] Diaz J, Schain RJ, Bailey BG. Phenobarbital-induced brain growth retardation in artificially reared rat pups. *Biol Neonate* 1977;**32**:77–82.

[4] Diaz J, Schain RJ. Phenobarbital: effects of long-term administration on behaviour and brain of artificially reared rats. *Science* 1978;**199**:90–1.

[5] Selhorst JB, Kaupman B, Horwitz JJ. Diphenylhydantoin-induced cerebellar degeneration. *Arch Neurol* 1972;**27** 453–5.

[6] Swaimann KF, Neale EA, Schrier BK, *et al.* Toxic effect of phenytoin on developing cortical neurones in culture. *Ann Neurol* 1983;**13**:48–52.

[7] Schiff D, Chan G, Stern L. Drug combinations and displacement of bilirubin from albumin. *Pediatrics* 1971;**48**:139–41.

[8] Engel R. *Abnormal electroencephalogram in the neonatal period.* Springfield: Thomas, 1975.

[9] Werner SS, Stockard JE, Bickford RG. *Atlas of neonatal electroencephalography.* New York: Raven Press, 1977.

[10] Tharp B, Cukier F, Monod N. The prognostic value of the electroencephalogram in premature infants. *Electroencephalogr Clin Neurophysiol* 1981;**51**:219–36.

[11] Monod N, Pajot N, Guidasci S. The neonatal EEG: statistical. *Electroencephalogr Clin Neurophysiol* 1972;**32**:529–44.

[12] Dreyfus-Brissac C. Neonatal electroencephalography. *Reviews in Perinatal Medicine* 1979;**3**:397–472.

[13] Connell JA, Oozeer RC, Dubowitz V. Continuous 4-channel EEG monitoring: a guide to interpretation, with normal values, in preterm infants. *Neuropediatrics* 1987;**18**:138–45.

[14] Dubowitz LMS, Dubowitz V. *The neurological assessment of the preterm and full-term infant. Clinics in developmental medicine, No 79.* London: Heinemann, 1981. (Spastics International Medical Publications.)

[15] Griffiths R. *The abilities of babies: a study in mental measurement.* Amersham: Association for Research in Infant and Child Development, 1976.

[16] Amiel-Tison C, Grenier A. *Evaluation neurologique du nouveau-né et du nourisson.* Paris: Masson, 1980.

[17] Touwen BLC. *Examination of the child with minor neurological dysfunction. Clinics in developmental medicine. No. 71.* Oxford: Blackwell, 1979. (Spastics International Medical Publications.)

[18] Drillien CM. Abnormal neurological signs in the first year of life in low birth-weight infants: possible prognostic significance. *Dev Med Child Neurol* 1972;**14**:575–84.

[19] Volpe JJ. Neonatal seizures. *Neurology of the newborn.* Philadelphia: Saunders, 1987:129–59.

[20] Purpura DP. Stability and seizure susceptibility. In: Jasper HH, Ward A, Pope A, eds. *Basic mechanisms of the epilepsies.* Boston: Little Brown, 1969:481–505.

[21] Freeman JM, Leitman PS. A basic approach to the understanding of seizures and the mechanism of action and metabolism of anticonvulsants. *Adv Pediatr* 1973;**20**:291.

[22] Dehkhargani F, Sarnat HB. Neonatal seizure. *Topics in neonatal neurology.* New York: Grune and Stratton, 1984:209–32.

[23] Eyre JA, Wilkinson AR. Thiopentone-induced coma after severe birth asphyxia. *Arch Dis Child* 1986;**61**:1084–9.

[24] Svenningsen NW, Blennow G, Lindroth M, Gaddlin PO, Ahlstrom H. Brain-orientated intensive care treatment in severe neonatal asphyxia. *Arch Dis Child* 1982;**57**:176–83.

[25] Staudt F, Scholl ML, Coen RW, Bickford RW. Phenobarbital therapy in neonatal seizures and the prognostic value of the EEG. *Neuropediatrics* 1982;**13**:24–33.

[26] Radvanyi-Bouvet MF, Vallecalle MH, Morel-Kahn F, Relier JP, Dreyfus-Brisac C. Seizures and electrical discharges in premature infants. *Neuropediatrics* 1985;**16**:143–8.

[27] Meldrum B. Possible therapeutic applications of antagonists of excitatory amino acid neurotransmitters. *Clin Sci* 1985;**68**: 113–22.

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Cerebral haemodynamic effects of changes in positive end expiratory pressure in preterm infants

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SUMMARY The effects of changes in positive end expiratory pressure (PEEP) on cerebral blood flow velocity, arterial blood gases, and mean arterial pressure were studied in newborn infants. In mechanically ventilated premature infants with severe respiratory disease, an increase in PEEP from 2 to 6 cm H_2O was associated with an increase in cerebral blood flow velocity. There was no significant change in mean arterial blood pressure. There was a significant increase in PaO_2 and $PaCO_2$ for every stepwise rise in PEEP. Multivariate regression analysis showed that 72% of the effect on cerebral blood flow velocity was attributable to $PaCO_2$ alone and that any change in blood pressure was not likely to contribute to these changes. There was no evidence that changes in PEEP within the commonly used range adversely affected the neonatal cardiovascular or cerebral circulations.

The introduction of intermittent positive pressure ventilation (IPPV) has led to a large increase in the number of premature infants that survive the neonatal period, but the risk of sustaining perinatal cerebral lesions is increased in infants who are treated by mechanical ventilation.[1] There has been considerable interest in the haemodynamic effects of IPPV in adults and ventilation can potentially influence the cerebral circulation in a number of ways, including by a direct effect on cardiac output,[2–7] by increasing intracranial pressure,[8 9] or by reducing cerebral venous return.[2 8]

There is little information about the effects of respiratory support on cerebral haemodynamics in newborn babies, and the present study was designed to investigate the effect of different positive end expiratory pressures on cerebral blood flow velocity.

Methods

The study was performed in the neonatal unit at the Leicester Royal Infirmary between April 1987 and January 1988. All the babies entered into the study had idiopathic respiratory distress syndrome and at the time of the study they were in the acute phase of their disease and showing no clear signs of improvement. All the infants had indwelling arterial catheters in place. An infant was excluded if a pneumothorax was present, if a cerebral ultrasound scan showed any abnormality, or if the clinical condition was unstable. None of the infants was receiving any drug known to influence the cerebral circulation. The Leicestershire district ethical committee approved this study and informed consent was obtained from the parents before all studies. The variables that were measured are given below.

CEREBRAL BLOOD FLOW VELOCITY

Recordings of cerebral blood flow velocity were made from the anterior cerebral artery using an ATL duplex Doppler system with a 724A probe (pulse echo 7·5 MHz, Doppler frequency 5·0 Mhz). The artery was visualised in the sagittal plane with the probe over the infant's anterior fontanelle. The Doppler sample volume was positioned in the straight portion of the artery, midway between the origin of the anterior cerebral artery at the internal carotid artery and the most inferior part of the corpus callosum. The sample volume was set to 3 mm and the high pass filter to 100 Hz. The signals from 25 to 30 consecutive cardiac cycles were recorded onto audiocassette tape and analysed off line through an Angioscan spectrum analyser. The maximum frequency envelope of the waveform was extracted and the velocity calculated from the wellknown Doppler equation. The artery was insonated at a point where the direction of blood flow was directly towards the transducer to minimise the error in the velocity calculation. The mean velocity over the cardiac cycle was derived by halving the maximum velocity.[10]

ARTERIAL BLOOD PRESSURE RECORDINGS

All the infants had direct arterial access either through an umbilical line positioned with its tip at the level of the diaphragm or through a radial line. The lines were attached by non-compliant tubing to a Gould pressure transducer with permanent write out onto a chart recorder. An observer recorded the mean arterial blood pressure immediately before the cerebral blood flow velocity recordings were made.

AIRWAY PRESSURE RECORDINGS

Ventilator pressures were recorded through a 19 gauge needle that was inserted into an endotracheal tube and then connected to a pressure transducer. The transducer signal was amplified and displayed using a Gould 4 channel recorder. Before each study the transducer was balanced and calibrated electronically.

It was thus possible to record pressure swings between peak inspiratory pressure and the positive end expiratory pressure (PEEP) directly at each stage of the study. In order to measure PEEP accurately it was necessary to open the ventilator circuit briefly; this permitted the pressure recorded by the transducer to drop to atmospheric.

Tidal volume was recorded so that the infants' respiratory pattern of interaction with the ventilator could be documented. This was done with a pneumotachograph (Gaeltec), integrator (Gould), and a differential pressure transducer (Gaeltec), in series with the ventilator. The pneumotachograph was placed between the endotracheal tube and the patient manifold of the ventilator. So that this addition to the ventilator circuit did not cause an unacceptable increase in the dead space, a flow of gas was created through the pneumotachograph as previously described.[11] Volume data obtained were used only qualitatively to determine patterns of interaction.

At each study 0·2 ml of blood was taken through the arterial line and the $PaCO_2$ and the PaO_2 tensions were determined.

PROCEDURE

The infants were studied at three PEEPs; low (2 cm H_2O), intermediate (4 cm H_2O), and high (6 cm H_2O). Finally the PEEP was reset at 2 cm H_2O and the pump in the pneumotachograph circuit was removed. This was done to maintain a comparatively high $PaCO_2$ so that a comparison could be made of the cerebral haemodynamics at the same PEEP but with different $PaCO_2$ tensions. The peak inspiratory pressure was not altered between the recordings. A period of 10 minutes was allowed for stabilisation after each change in PEEP before a sequence of measurements was made. After each manoeuvre measurements were made of cerebral blood flow velocity, mean intra-arterial blood pressure, respiratory pattern, the peak inspiratory pressure and PEEP, as well as estimates of arterial $PaCO_2$ and PaO_2 tensions. At the end of the study the ventilator settings were returned to the positions they had been in before the study.

STATISTICAL ANALYSIS

The significance of differences between physiological variables at the three PEEPs were tested by the paired *t* test. No adjustment of nominal p value was made for multiple comparisons in view of the small number of groups compared.

Table *Details of 17 infants studied, peak inspiratory pressure, and use of pancuronium at the time of study*

Case No	*Gestation (weeks)*	*Birthweight (g)*	*Age (hours) at time of study*	*Peak inspiratory pressure (mm Hg)*	*Pancuronium used during study*
1	28	910	108 173	17·7, 17·7	No, no
2	31	1520	15 108	25·8, 17·0	Yes, no
3	28	1330	100 173	21·4, 19·7	Yes, yes
4	27	990	329	20·0	No
5	25	860	9	20·0	No
6	33	1570	120	34·6	No
7	29	1500	49	31·0	Yes
8	28	1090	25	21·7	No
9	31	1830	16	28·5	No
10	31	1280	68	16·0	Yes
11	27	730	98	22·0	No
12	32	1860	96	21·8	No
13	32	1470	85	25·0	Yes
14	24	820	74	18·4	No
15	35	2320	46	29·2	Yes
16	29	1080	12	25·7	No
17	37	4400	92	29·9	No

The dependence on cerebral blood flow velocity on $PaCO_2$, PaO_2, and mean arterial blood pressure was examined by multivariate regression. The suitability of the linear model was confirmed by the use of residual plots. The additional effect of PEEP was then examined by inclusion of dummy variables representing different PEEPs.

Results

Seventeen infants were studied on 20 occasions. The median gestation was 28·5 weeks (range 24–37) and the median birth weight was 1330 g (range 730 to 4400). The median age at the time of the study was 88·5 hours (range 9 to 329). Seven studies were performed while the infants were receiving pancuronium for neuromuscular paralysis. Details of the infants are shown in the table.

The PEEP measured directly from the airway differed in some cases from the ventilator manometer dial. The actual median PEEP at the low level (n=17) was 2·2 cm (range 0 to 6), at the intermediate level 4 cm (range 2·4 to 6·1), and at the high level 6·8 cm (range 3·4 to 8·5). Despite these variations every infant showed a sequential increase in the particular level of PEEP as planned.

The changes in PaO_2 and $PaCO_2$ with increasing levels of PEEP are shown in fig 1. The mean (SEM) increase in the $PaCO_2$ between the studies at low and intermediate PEEP was 0·7 (0·17) kPa, and between low and high PEEP was 1·3 (0·27) kPa. These changes were significant ($p<0·001$ on both occasions). The mean increase in $PaCO_2$ between the studies at 2 cm of PEEP with and without the pump was 1·2 (0·23) kPa ($p<0·001$).

The mean increase in PaO_2 between low and intermediate PEEP was 0·9 (0·41) kPa, and between low and high PEEP 2·8 (0·74) kPa. These changes were significant ($p<0·04$ and $<0·002$, respectively). There was a significant increase of 1·6 (0·53) kPa in the PaO_2 between the studies at 2 cm with and without the pump ($p<0·01$).

The changes in cerebral blood flow velocity and mean arterial blood pressure with different levels of PEEP are shown in fig 2. The mean increase in cerebral blood flow velocity from the anterior cerebral artery between the studies at low and intermediate PEEP was 2·1 (0·81) cm/s, and between low and high PEEP 4·2 (1·00) cm/s. The increase was significant on both occasions ($p<0·025$ and $<0·001$, respectively). The mean increase between the studies at low PEEP with and without the pump was 3·4 cm/s (0·92) ($p<0·005$).

The mean increase in arterial blood pressure between low and intermediate PEEP was 1·2 (1·07) mm Hg. The increase in arterial blood pressure

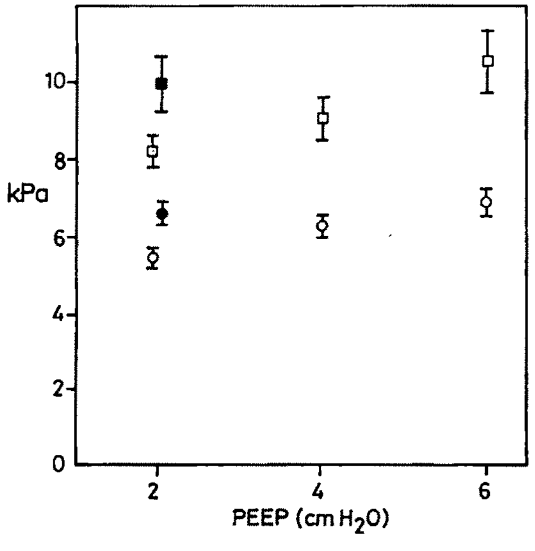

Fig 1 *Effect of PEEP with (open symbols) and without (closed symbols) the pump in circuit on PaO_2 (squares) and $PaCO_2$ (circles). Points are means (SEM).*

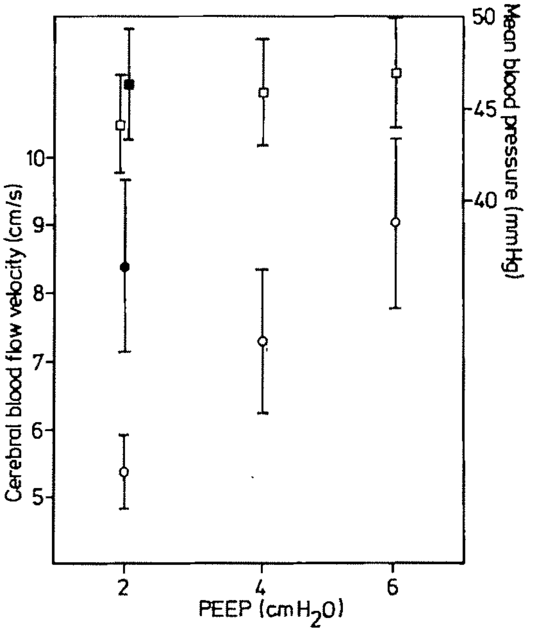

Fig 2 *Effect of PEEP with (open symbols) and without (closed symbols) the pump in circuit on mean arterial blood pressure (squares) and cerebral blood flow velocity. Points are means (SEM).*

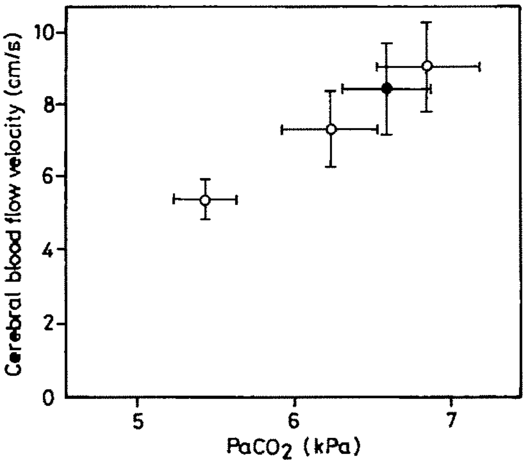

Fig 3 *Association between cerebral blood flow velocity and* $PaCO_2$*. Points are means (SEM) and represent readings at three settings of PEEP with (open circles) and without (closed circles) pump in circuit.*

between low and high PEEP was 1·8 (1·52) mm Hg, and between the studies at low PEEP with and without the pump was 2·25 (1·17) mm Hg. None of these changes was significant.

A multivariate regression analysis was carried out to investigate the dependence of cerebral blood flow velocity on the potential explanatory variables: $PaCO_2$, PaO_2, blood pressure, and PEEP. The analysis showed that there was a significant association between cerebral blood flow velocity and $PaCO_2$ ($p<0{\cdot}001$) and PaO_2 ($p<0{\cdot}02$). Of the variation in cerebral blood flow velocity, 80% was attributable to the changes in $PaCO_2$ and PaO_2 (R-sq adjusted=80%), and 71·5% of the variation was attributable to $PaCO_2$ alone. There was no improvement in the model when blood pressure was incorporated. The coefficient of mean arterial blood pressure when added to $PaCO_2$ and PaO_2 was not significant ($p=0{\cdot}7$). The inclusion of PEEP in the multivariate analysis did not improve the explanatory model ($p>0{\cdot}9$) and we concluded that an increase in PEEP between the levels of 2 and 6 cm did not have a direct effect on the cerebral circulation.

The pneumotachograph recordings showed the pattern of interaction between the infants' spontaneous breaths and the ventilators in those infants who were not paralysed. In none of these infants did the pattern change significantly among the settings of PEEP, and we therefore assumed that the infants' arousal states had not changed significantly between the studies.

Discussion

We have shown that increasing PEEP to 6 cm H_2O causes a rise in cerebral blood flow velocity, and it is likely that this change is mediated by alterations in blood gas tensions. Doppler assessments of cerebral haemodynamics measure the velocity of blood flow and not volumetric flow, but a number of investigators have shown a close correlation between cerebral blood flow velocity and volumetric assessment of cerebral blood flow in newborn animals[12 13] and in human infants.[14] We believe, therefore, that cerebral blood flow increased when PEEP was increased between these settings.

There have been few studies of the effects of changes in IPPV on the cerebral circulation in human infants. Ellison and Denny[15] found that the mean cerebral blood flow velocity was significantly reduced in infants receiving ventilation, and Cowan and Thoresen[16] found that the peak inspiratory pressure affected the Doppler arterial waveform in a proportion of newborn infants who required ventilation. Leahy *et al*[17] showed that an increase in cranial blood volume occurred in infants who required mechanical ventilation compared with those that were breathing spontaneously.

We have shown that cerebral blood flow velocity increases as PEEP increases. From the multivariate analysis it seems that the increase in $PaCO_2$ between the levels of PEEP was the most important factor in determining the cerebral blood flow velocity. This is perhaps not surprising, as carbon dioxide is a potent stimulator of cerebral blood flow in premature infants.[18] We have previously reported that increases in $PaCO_2$ cause pronounced increases in cerebral blood flow velocity even in the most immature infants.[19] and that hyperoxia also produces increases in cerebral blood flow velocity though to a lesser extent.[20]

An increase in the airway pressure can compromise cardiac function in a number of ways. Morgan *et al*[2] concluded that the most important haemodynamic effects of positive pressure ventilator treatment were an increase in the intrathoracic pressure with a reduction in venous return to the heart. Aidinis *et al*[8] thought that this increase in intrathoracic pressure would increase intracranial pressure and reduce systemic blood pressure. The combination of these effects would reduce cerebral perfusion pressure and have potentially disastrous consequences for premature infants' brains. Cardiac function could be further compromised by increases in pulmonary vascular resistance as the alveoli become distended by increasing levels of PEEP, this would increase the resistance to blood flow within the lungs, and cause reduced left ventricular dimensions by over-loading the volume in the right ventricle.[4] Although we did not measure cardiac output we did not find a significant fall in the blood pressure as PEEP was increased, and our results suggest that levels of PEEP used in the management of respiratory illness in neonates do not produce serious functional effects on cardiac output.

When considering the effects of PEEP we have analysed the results according to whether the level was low, intermediate, or high, and we have looked at the change in the variables between the settings. We have not considered absolute levels of PEEP as it is clear that the effect of PEEP on the cardiovascular or cerebral circulations in any particular infant will be greatly modified by the compliance of the lung.[6] The pressure transducer connected to the infant's airway did, however, enable us to check that increasing the PEEP settings on the ventilator did result in an increase in the airway pressure. Unfortunately it was not possible to measure lung compliance and we accept that these conclusions may not be relevant to all infants with lung disease. Most of the infants had moderate to severe respiratory distress syndrome and presumably poorly compliant lungs, and hence were comparatively resistant to alveolar distension resulting from increases in airway pressure.

In conclusion, increasing levels of PEEP cause significant increases in arterial blood gas tensions, and it is these changes that increase cerebral blood flow velocity. There is no evidence that levels of PEEP up to 6 cm H_2O reduce cerebral blood flow.

References

[1] Levene MI, Fawer C-L, Lamont RF. Risk factors in the development of intraventricular haemorrhage in the preterm neonate. *Arch Dis Child* 1982;**57**:410–7.

[2] Morgan BC, Martin WE, Hornbeam TF, Crawford EW, Guntheroth WG. Haemodynamic effects of intermittent positive pressure ventilation. *Anaesthesiology* 1966;**27**:584–90.

[3] Qvist J, Pontoppidan H, Wilson RS, Lowenstien E, Laver MB. Haemodynamic responses to mechanical ventilation with PEEP. *Anesthesiology* 1975;**42**:45–55.

[4] Jardin F, Farcot JC, Boisante L, Curien N, Margairaz A, Bourdarias JP. Influence of positive end expiratory pressure on left ventricular performance. *N Engl J Med* 1981;**304**:387–92.

[5] Santamore WP, Heckman JL, Bove AA. Cardiovascular changes from expiration to inspiration during IPPV. *Am J Physiol* 1983;**245**:H307–12.

[6] Mirro R, Busija D, Green R, Leffler C. Relationships between airway pressure, cardiac output and organ blood flow with normal and decreased respiratory compliance. *J Pediatr* 1987;**111**:101–6.

[7] Hausdorf G, Hillwege HH. Influence of positive end-expiratory pressure on cardiac performance in premature infants: A Doppler-echocardiographic study. *Crit Care Med* 1987;**15**: 661–4.

[8] Aidinis SJ, Laferty J, Shapiro HM. Intracranial responses to PEEP. *Anesthesiology* 1976;**45**:275–86.

[9] Huseby JS, Pavlin EG, Butler J. Effect of positive end expiratory pressure on intracranial pressure in dogs. *J Appl Physiol* 1978;**44**:25–7.

[10] Evans DH. On the measurement of mean velocity of blood over the cardiac cycle using Doppler ultrasound. *Ultrasound Med Biol* 1985;**11**:735–41.

[11] Field D, Milner AD, Hopkin IE. High and conventional rates of positive pressure ventilation. *Arch Dis Child* 1984;**59**:1151–4.

[12] Batton DG, Hellmann J, Hernandez MJ, Maisels MJ. Regional blood flow cerebral blood velocity and pulsatility index in newborn dogs. *Pediatr Res* 1983;**17**:908–12.

[13] Hansen NB, Stonestreet BS, Rosenkrantz TS, Oh W. Validity of Doppler measurements of anterior cerebral artery blood flow velocity: correlation with brain blood flow in piglets. *Pediatrics* 1983;**72**:526–31.

[14] Grieson G, Johnsen K, Ellison PH, Fredriksen PS, Mali J, Friis-Hanson B. Cerebral blood flow in the newborn infant: comparison of Doppler ultrasound and 133 Xenon clearance. *J Pediatr* 1984;**104**:411–7.

[15] Ellison P, Denny J. Doppler measures of cerebral blood flow: respirator settings and blood gases in the neonatal intensive care unit. *Ann Neurol* 1984;**12**:216–7.

[16] Cowan F, Thoresen M. The effects of intermittent positive pressure on the cerebral arterial and venous velocities in the newborn infant. *Acta Paediatr Scand* 1987;**76**:239–47.

[17] Leahy FAN, Cates D, MacCallum M, Rigato H. Effect of CO_2 and 100% 02 on cerebral blood flow in preterm infants. *J Appl Physiol* 1980;**48**:468–72.

[18] Greisen G, Trojaborg W. Cerebral blood flow, $PaCO_2$ changes and visual evoked potentials in mechnically ventilated, preterm infants. *Acta Paediatr Scand* 1987;**76**:394–400.

[19] Levene MI, Shortland D, Gibson N, Evans DH. Carbon dioxide reactivity of the cerebral circulation in extremely premature infants: effects of postnatal age and indomethacin. *Pediatr Res* 1988;**24**:175–9.

[20] Niijima S, Shortland DB, Levene MI, Evans DH. Transient hyperoxia and cerebral blood flow velocity in infants born prematurely and at full term. *Arch Dis Child* 1988;**63**:1126–30.

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Electrolytes and glucose in cerebrospinal fluid of premature infants with intraventricular haemorrhage: role of potassium in cerebral infarction

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SUMMARY Potassium, sodium, and glucose concentrations in cerebrospinal fluid and plasma were determined in 73 infants whose gestational ages ranged from 25 to 40 weeks. Six of 29 (21%) neonates with intraventricular haemorrhages had raised potassium concentrations in the cerebrospinal fluid (3·7–30 mmol/l); five developed cerebral infarctions. There was a significant correlation between sodium and glucose concentrations in plasma and cerebrospinal fluid with prolonged hypoglycorrhachia accompanying posthaemorrhagic hydrocephalus. Raised concentrations of potassium in cerebrospinal fluid occur with intraventricular haemorrhage and may contribute to the development of cerebral infarction.

The increased incidence of cerebral infarction in preterm infants with intraventricular haemorrhage has been well described[1] and may result from common aetiological factors. There is, however, circumstantial evidence that intraventricular haemorrhage may lead to infarction. The median time of onset of cerebral infarction is reported to be significantly later than that of intraventricular haemorrhage. Cerebral vasospasm occurs after subarachnoid haemorrhage in adults and may lead to haemorrhagic infarction, thereby causing considerable morbidity and mortality.[2 3] In premature infants with intraventricular and intracerebral haemorrhages, a pronounced reduction in regional cerebral blood flow has been shown on positron emission tomography.[4]

Vasoactive compounds are released after the lysis of red cells and platelets, and these are capable of causing vasospasm in vitro. Oxyhaemoglobin, prostaglandins, and lipid hydroperoxides have all been implicated in producing vasoconstriction of cerebral vessels.[5] Edvinsson *et al* showed that high concentrations of potassium in the cerebrospinal fluid cause pronounced constriction of isolated human pial arterioles and small arteries.[6] Concentrations sufficient to produce this effect were attained following lysis of blood clot in simulated cerebrospinal fluid. They proposed that high concentrations of potassium in the cerebrospinal fluid may play a part in the development of infarction accompanying intraventricular haemorrhage in neonates by inducing vasospasm in the surrounding cerebral tissue.

The aim of this study was to measure the concentrations of potassium in the cerebrospinal fluid of preterm infants who had had intraventricular haemorrhages, to determine whether high concentrations occur in vivo, and in addition to examine the effect of intraventricular haemorrhage on concentrations of sodium and glucose in the cerebrospinal fluid. The presence of ultrasound changes compatible with cerebral infarction was recorded.

Patients and methods

Neonates requiring lumbar puncture as part of a screen for infection were entered into the study. In addition to the cerebrospinal fluid obtained for culture, 0·5–1 ml was collected in a plain tube and centrifuged immediately to remove any intact red cells that may have been introduced by the trauma of the lumbar tap. The supernatant sodium, potassium, and glucose concentrations were measured in the cerebrospinal fluid as well as in heparinised and fluorinated blood samples obtained at the time of lumbar puncture. To minimise the risk of haemolysis, blood was obtained from a flushed umbilical arterial catheter or peripheral vein. The presence of haemolysis was reported by the laboratory staff when they had looked at the plasma.

Plasma and cerebrospinal fluid sodium and potassium concentrations were measured directly using ion selective electrodes (Corning 614). Potassium concentrations of greater than 10 mmol/l were determined by flame photometer (IL 543). Plasma and cerebrospinal fluid glucose concentrations were measured by the glucose oxidase oxygen consumption method (Beckman glucose analyser). The coefficient of variation for each of the above methods was 0·5%, 2%, and 5%, respectively.

Cranial ultrasound scans were carried out on preterm infants of less than 34 weeks' gestation daily for seven days, then one to three times/week, with a real time sector scanner with 7·5 mHz transducer. More mature infants had ultrasound scans at the time of the lumbar puncture and as indicated thereafter. The patients were divided into groups according to the cranial ultrasound scan findings. Periventricular haemorrhage was graded according to the following simple classification: grade I—subependymal haemorrhage, grade II—intraventricular haemorrhage, and grade III—parenchymal echodensity adjacent to the ventricles.

Biochemical data within groups were analysed by the Pearson rank correlation test, and between groups by the Mann-Whitney U test.

Results

Concentrations of potassium, sodium, and glucose in cerebrospinal fluid and plasma were measured on 68 occasions in 57 neonates aged 1–14 days, of 25–40 weeks' gestation. Thirty one samples of cerebrospinal fluid were also obtained from 28 neonates of 28–40 weeks' gestation (median 32), who had normal ultrasound scans. These constitute the control group. Ultrasound changes compatible with intraventricular haemorrhage grade I–III were present in 29 neonates of 25–34 weeks' gestation (median 28), who formed the intraventricular haemorrhage group. All echodense intracerebral lesions that were seen had occurred in association with intraventricular haemorrhages.

The cerebrospinal fluid potassium concentrations in patients in the control group ranged from 2·8–3·4 mmol/l, mean (SD) 3·1 (0·15) mmol/l. There was no significant correlation between the cerebrospinal fluid and plasma potassium concentrations (r=0·14) (fig 1); the cerebrospinal fluid potassium was maintained within a narrow range. Dividing the patients in the control group according to gestational ages of 25–29 weeks, 30–34 weeks, and 35–40 weeks, no significant differences were found in the cerebrospinal fluid potassium concentrations among the groups (mean (SD)) cerebrospinal fluid potassium concentration 3·15 (0·14) mmol/l, 3·09 (0·18), and 3·13 (0·1) mmol/l, respectively) indicating that the cerebrospinal fluid potassium concentration is as well controlled in the immature as in the mature infants.

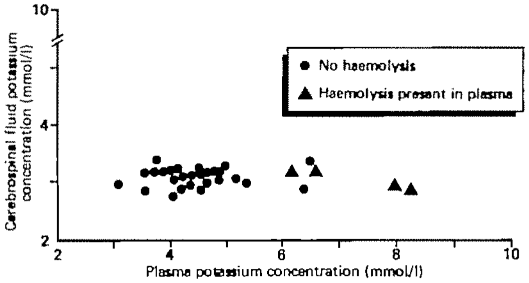

Fig 1 *Cerebrospinal fluid and plasma potassium concentrations in neonates aged <14 days who did not develop intraventricular haemorrhages.*

The cerebrospinal fluid potassium concentration in the group with intraventricular haemorrhage ranged from 2·6 to 30 mmol/l, mean (SD) 4·2 (4·5) mmol/l. Six neonates had cerebrospinal fluid potassium concentrations two standard deviations above the mean in the control group, with potassium concentrations ranging from 3·7–30 mmol/l (median 6·6) (fig 2 and table 1). Case 1 developed intracerebral echodense areas 24 hours after lumbar puncture. The intracerebral echodense areas in cases 1–3 became more extensive 24–96 hours after lumbar puncture.

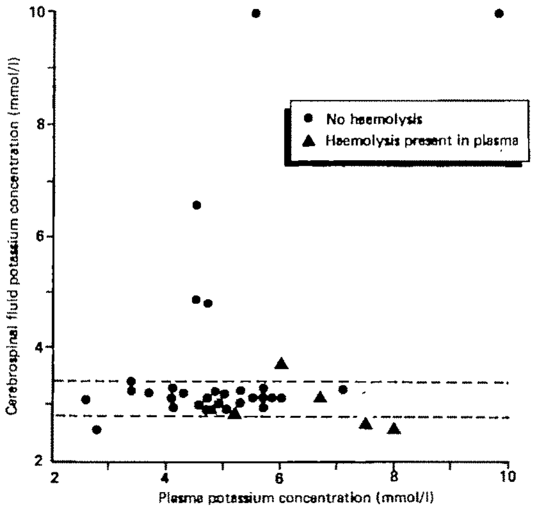

Fig 2 *Cerebrospinal fluid and plasma potassium concentrations in neonates aged <14 days who had intraventricular haemorrhages. The two points at the top of the figure represent a cerebrospinal fluid potassium concentration of (left) >10 mmol/l and (right) 30 mmol/l.*

Table *Details of six patients with intraventricular haemorrhage and cerebrospinal fluid potassium concentrations more than two standard deviations above the mean*

Gestation (weeks)	*Age (days)*	*Potassium concentration cerebrospinal fluid (mmol/l)*	*Cranial ultrasound scan at time of lumbar puncture*	*Subsequent ultrasound appearance*
27	<1	4·8	Bilateral, grade II	Porencephaly
28	1	6·6	Right, grade III	Hydrocephaly
			Left, grade II	Porencephaly
26	2	4·9	Left, grade III	Hydrocephaly Porencephaly
25	4	30	Bilateral, grade III	Died
25	6	3·7	Left, grade III	Porencephaly
28	7	>10	Bilateral, grade II	Normal

Cerebrospinal fluid was obtained from the neonates with high cerebrospinal fluid potassium concentrations at the age of 1–7 days (median 2). Ultrasonographic changes of infarction were present from the ages of 1–5 days (median 2). A further six patients with intraventricular haemorrhage and ultrasonographic changes compatible with infarction had normal cerebrospinal fluid potassium concentrations at the time of cerebrospinal fluid sampling. The cerebrospinal fluid was, however, obtained later, at 1–13 days (median 10) with areas of intracerebral echodensities appearing from the ages of 2–4 days (median 2); high cerebrospinal fluid potassium concentrations may have occurred at an earlier stage. On follow up ultrasound scans all had developed intracerebral cystic changes, and the intracerebral echodensities were contiguous with the ventricular echodensity. These were compatible with ultrasound appearances of haemorrhagic infarction or extension of intraventricular haemorrhage. On follow up scans the intracerebral echodense areas had resolved with cystic formation confirming that destruction of brain tissue (infarcion) had occurred.

The mean (SD) cerebrospinal fluid sodium concentration in the control group was 134 (3) mmol/l and in the intraventricular haemorrhage group 133 (5·5) mmol/l (p=0·4) with a mean cerebrospinal fluid:plasma sodium ratio of 0·99. There was a significant correlation between cerebrospinal fluid and plasma sodium in the two groups, r being 0·7 and 0·57, respectively. The mean cerebrospinal fluid:plasma sodium ratio was 0·99 (0·02) in the control group and 0·99 (0·04) in the intraventricular haemorrhage group.

The cerebrospinal fluid glucose concentrations in the normal group (range 0·5–8·1 mmol/l, median 3) correlated significantly (r=0·7) with the plasma concentrations (range 0·5–10 mmol/l, median 4·2) with a mean cerebrospinal fluid:plasma glucose ratio of 0·8 (range 0·34–2·6). A significant correlation (r=0·8) was also found between cerebrospinal fluid glucose (range 0·5–15·1 mmol/l, median 2·8) and plasma glucose (range 1·1–27 mmol/l, median 4·4) concentrations in the intraventricular haemorrhage group, with a mean cerebrospinal fluid:plasma glucose ratio of 0·6 (range 0·08–2). The lowest ratios were associated with the more extensive haemorrhages. Culture of cerebrospinal fluid was negative in all cases.

Thirteen samples of cerebrospinal fluid were obtained from 10 patients aged 15–58 days (gestation 25–28 weeks) who developed posthaemorrhagic hydrocephalus. Cerebrospinal fluid was removed at intervals before insertion of a ventriculoperitoneal shunt. Cultures of cerebrospinal fluid were negative in all cases. The cerebrospinal fluid glucose concentrations were significantly lower (p<0·001) in this group of patients than in the control or intraventricular haemorrhage groups, with a mean cerebrospinal fluid:plasma glucose ratio of 0·32, range 0·13–0·6 (fig 3). Two infants had raised cerebrospinal fluid potassium concentrations. One baby aged 17 days (gestation 28 weeks) had a large intraventricular clot with cerebral infarction on ultrasound scan. The ventricular cerebrospinal fluid potassium concentration was more than 10 mmol/l. Unfortunately the actual concentration was not determined. A baby of 26 weeks' gestation aged 58 days developed a haemorrhagic infarction in an area of porencephaly after repeated ventricular taps for non-communicating hydrocephalus. The ventricular cerebrospinal fluid potassium concentration was 7·6 mmol/l.

Cerebrospinal fluid was obtained from a further six patients aged 14–102 days (gestation 25–35 weeks) at the time of infection screen. All had normal cranial ultrasound scans throughout. The cerebrospinal fluid:plasma glucose ratio in this group ranged from 0·4 to 1·2, mean 0·7 (0·2). All but one had cerebrospinal fluid potassium concentrations within the range of the control group. On day 27 one infant of 25 weeks' gestation had a cerebrospinal fluid potassium concentration of 9 mmol/l (plasma concentration 5·3), and on day 34 a cerebrospinal fluid concentration of 4·3 mmol.

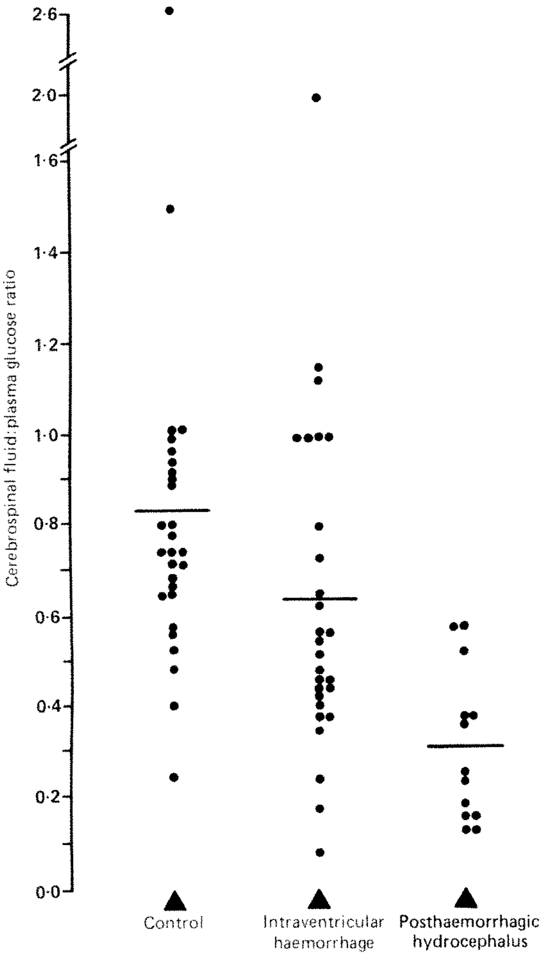

Fig 3 *Ratio of cerebrospinal fluid:plasma glucose in the three groups of babies.*

The sodium cerebrospinal fluid:plasma ratio was 0·98 (0·04) and 0·99 (0·008) in the posthaemorrhagic hydrocephalus group and the last group without intraventricular haemorrhage, respectively.

Discussion

Cerebrospinal fluid potassium concentration in adults and various animals (for example, rabbits, cats, dogs, and goats) is controlled within the narrow range 2·5–3·3 mmol/l despite wide variations in plasma potassium concentrations.[7 8] There is evidence that the maintenance of the cerebrospinal fluid potassium, with increased efflux of this ion in the presence of raised concentrations, depends on an active sodium/potassium pump.[8] This control is maintained despite the association of various diseases (for example, tuberculosis, meningitis, hypertension, and encephalopathy). Cooper *et al* did not find alterations in cerebrospinal fluid potassium concentrations in adult patients with cerebral haemorrhages despite the presence of large amounts of blood in the subarachnoid space.[9] We found that the mechanisms for maintaining cerebrospinal fluid potassium concentrations are established in neonates even at 26 weeks' gestation, the cerebrospinal fluid potassium ranging from 2·8–3·4 mmol/l irrespective of the plasma concentration. In the presence of intraventricular blood, however, raised cerebrospinal fluid potassium concentrations were found.

Six of 30 of the neonates (20%) aged less than 14 days who had intraventricular haemorrhages on ultrasound scan had cerebrospinal fluid potassium concentrations two standard deviations outside the mean for our control population (median 6·6 mmol/l, range 3·7–30). The cerebrospinal fluid potassium concentrations were recorded mainly in specimens obtained at lumbar puncture. These concentrations have been shown to be lower than ventricular concentrations. The most relevant cerebrospinal fluid potassium concentration is that measured adjacent to the ventricular blood clot at the site of release from haemolysing red cells. This study was limited, as cerebrospinal fluid was only obtained when there was a clinical indication for lumbar puncture. Ideally the cerebrospinal fluid concentrations should be measured daily to detect any transient rise that may occur with peak haemolysis. After the addition of blood to simulated cerebrospinal fluid in vitro in concentrations of 10, 30, and 50%, peak cerebrospinal fluid potassium concentrations 6, 16, and 24 mmol/l were obtained, respectively by the fourth day.[6] With small intraventricular haemorrhages the homoeostatic mechanisms concerned in the control of the cerebrospinal fluid potassium concentration may be adequate, but with large volumes these may be overwhelmed leading to high circulating cerebrospinal fluid potassium concentrations that are detectable by lumbar puncture.

Five of 11 patients (45%) who had evidence of cerebral infarction on ultrasound scans had raised cerebrospinal fluid potassium concentrations. These did not represent the peak concentrations achieved, but are random samples as already discussed. A raised cerebrospinal fluid potassium concentration was recorded in only one patient with grade II periventricular haemorrhage 24 hours before the appearance of intracerebral echodensity adjacent to and contiguous with the ventricle. The other four infants already had grade III periventricular haemorrhages at the time of their lumbar punctures. Intracerebral echodensity was not recorded in the baby of 28 weeks' gestation who had a cerebrospinal fluid potassium concentrations of more than 10 mmol/l at 7 days.

The intracerebral echodense areas represent either haemorrhage into infarcted tissue or venous infarction secondary to haemorrhage, as described by Gould *et al.*[10] The presence of infarction is supported by the development of cystic lesions in those who survived.

Two further patients with posthaemorrhagic hydrocephalus had raised potassium concentrations, one of whom had had a recent haemorrhage into an area of infarct. Edvinsson *et al* showed that there was pronounced vasoconstriction of pial and cerebral blood vessels when potassium concentrations were greater than 10 mmol/l.[6] They proposed that potassium affects vascular smooth muscle through the depolarisation of the cell membrane and the opening of potential operated calcium channels. High concentrations of extracellular potassium result in a large increase in cerebral glucose utilisation associated with a spread of intense neuronal activity followed by membrane depolarisation and electrical depression.[11] Edvinsson *et al* concluded that a high cerebrospinal fluid potassium concentration produced by haemolysing red cells from an intraventricular haemorrhage may induce vasospasm in the surrounding cerebral tissue in addition to increasing its energy needs. In this way intraventricular haemorrhage may lead to cerebral infarction.

In our study a raised cerebrospinal fluid potassium concentration was present in one patient with intraventricular haemorrhage before the appearance of echodensity in the cerebral tissue. Evidence of cerebral infarction or haemorrhage, or both, was already present at the time of the lumbar punctures in the other neonates who had raised cerebrospinal fluid potassium concentrations. Within the limitations of this study we did not demonstrate that raised cerebrospinal fluid potassium concentrations lead to cerebral infarction, but did find that raised cerebrospinal fluid potassium concentrations do occur in premature infants with intraventricular haemorrhage in contrast with adults;[9] and the possibility remains that this may contribute to the development of infarction.

Extracellular potassium plays an important part in the regulation of neuronal excitability and it can therefore be expected that small changes in the cerebrospinal fluid potassium concentration may produce profound changes in the neurological and clinical state. The irritability and hypertonicity seen in some infants with intraventricular haemorrhage may be produced in this way, but we did not study this.

The cerebrospinal fluid sodium concentrations correlated with those in plasma, maintaining an osmotic equilibrium even in the presence of an intraventricular haemorrhage. Cooper *et al,* however, found a significant increase in the cerebrospinal fluid sodium concentrations in adult patients who had had cerebral haemorrhage.[9]

The cerebrospinal fluid glucose concentrations correlated with those in plasma, with a mean cerebrospinal fluid:plasma glucose ratio of 0·8 in the control group A cerebrospinal fluid:plasma glucose ratio of 0·4 represents the lower limit of normal.[12] Babies with the changes of extensive haemorrhage had the lowest ratios and the cerebrospinal fluid concentrations of glucose were low. For those who developed hydrocephalus the ratio remained low, with low white cell counts for up to 57 days although on ultrasound scan the blood clot had resolved. The reported gradual development of low cerebrospinal fluid glucose concentrations that reaches a trough when the number of viable cells in the cerebrospinal fluid is low with a slow return to normal,[13] supports the hypothesis that alteration or inhibition of the glucose transport system occurs by breakdown products of blood. Hypoglycorrhachia may persist for weeks, as shown in our study. No association has been found between the severity of intraventricular haemorrhage and the persistence of blood on computed tomography, the development of hydrocephalus,[14] or neurological outcome.[15]

The mechanisms of control of cerebrospinal fluid electrolytes and glucose are present in the most immature infant but may be disturbed by intraventricular haemorrhage. When intraventricular blood haemolyses, high cerebrospinal fluid potassium concentrations do occur, and may contribute to the development of cerebral infarction as a result of vasospasm.

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References

[1] Sinha SK, Davies JM, Sims DG, Chiswick ML. Relation between periventricular haemorrhage and ischaemic brain lesions diagnosed by ultrasound in very preterm infants. *Lancet* 1985;**ii**:1154–6.

[2] Fisher CM, Kistler JP, Davis JM. Relation of cerebral vasospasm to subarachnoid haemorrhage visualised by computerised tomographic scanning. *Neurosurgery* 1980;**6**:1–8.

[3] Saito I, Ueda Y, Sano K. Significance of vasospasm in the treatment of ruptured intracranial aneurysms. *J Neurosurg* 1977;**47**:412–29.

[4] Volpe JJ, Herscovitch P, Perlman JM, Raichle ME. Positron emission tomography in the newborn: extensive impairment of regional cerebral blood flow with intraventricular haemorrhage

and haemorrhagic intracerebral involvement. *Pediatrics* 1983; **72**:589–601.

[5] Sasaki T, Asano T, Takakura K, Sano K, Kassell NF. Nature of the vasoactive substance in the CSF from patients with subarachnoid haemorrhage. *J Neurosurg* 1984;**60**:1186–91.

[6] Edvinsson L, Lou HC, Tvede K. On the pathogenesis of regional cerebral ischemia in intracranial hemorrhage: a causal influence of potassium? *Pediatr Res* 1986;**20**:478–80.

[7] Bradbury MWB, Stubbs J, Hughes IE, Parker P. The distribution of potassium, sodium, chloride and urea between lumbar cerebrospinal fluid and blood serum in human subjects. *Clin Sci* 1963;**25**:97–105.

[8] Katzman R, Pappius HM. *Brain electrolytes and fluid metabolism.* Baltimore: Williams and Wilkins, 1973:80–95.

[9] Cooper ES, Lechner E, Bellet S. Relation between serum and cerebrospinal fluid electrolytes under normal and abnormal conditions. *Am J Med* 1955;**18**:613–21.

[10] Gould SJ, Howard S, Hope PL, Reynolds EOR. Periventricular intraparenchymal cerebral haemorrhage in preterm infants: the role of venous infarction. *J Pathol* 1987;**151**:197–202.

[11] Shinohara M, Dollinger B, Brown G, Rapoport S, Sokoloff L. Cerebral glucose utilisation: local changes during and after recovery from spreading cortical depression. *Science* 1979;**203**: 188–90.

[12] Donald PR, Malan C, Van der Walt A. Simultaneous determination of cerebrospinal fluid glucose and blood glucose concentrations in the diagnosis of bacterial meningitis. *J Pediatr* 1983;**103**:413–5.

[13] Mathew OP, Bland HE, Pickens JM, James EJP. Hypoglycorrhachia in the survivors of neonatal intracranial haemorrhage. *Pediatrics* 1979;**63**:851–4.

[14] Mathew OP, Volpe JJ. Neonatal intraventricular haemorrhage: hypoglycorrhachia and its relationship to CSF lactate levels. *J Pediatr* 1980;**97**:292–5.

[15] Th Deonna A, Calame A, Van Melle G, Prod'hom S. Hypoglycorrhachia in neonatal intracranial haemorrhage. Relationship to post haemorrhagic hydrocephalus. *Helv Paediatr Acta* 1977;**32**:351–61.

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British Association of Perinatal Medicine

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1990 Annual Meeting

13–15 September 1990, University Hospital of Wales.
Organiser: Dr Mark Drayton, Department of Child Health, University Hospital of Wales, Heath Park, Cardiff CF4 4XW.

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Doppler analysis of superior mesenteric artery blood flow in preterm infants

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SUMMARY Transcutaneous Doppler ultrasound measurements were made of the superior mesenteric artery of 22 preterm infants (mean birth weight 600–2580 g) to investigate the response of intestinal blood flow to feeding. Analysis of the data in relation to milk intake showed a significant change in peak velocity and mean velocity; peak velocity before feeding increased from a mean baseline value of 34 cm/s in fasting infants to 46 cm/s when less than 50 g milk/kg body weight a day were given, and up to 56 cm/s when 50 g or more of milk/kg body weight a day were given. The corresponding values for mean velocity were 10, 15, and 22 cm/s, respectively. The pulsatility index decreased from 0·94 to 0·90 and 0·86 as milk volumes were increased. This investigation showed characteristic changes in the response of intestinal blood flow to feeding: 15 minute peak velocity and mean velocity rose significantly 15, 45, and 90 minutes after feeding, whereas the pulsatility index fell. Flow rate peaked 45 minutes after feeding.

Several methods have been used to study blood flow of human intestine.[1] Only the transcutaneous Doppler flow method, however, has become a routine procedure for the evaluation of intestinal circulation. Recently there have been single reports of the possibilities of duplex scan examinations of the superior mesenteric artery in adults.[2 3] and a Doppler study of superior mesenteric artery blood flow measurements in healthy newborns has been carried out.[4] With the exception of a short abstract,[5] I know of no other investigation on intestinal circulation in preterm or infants born at full term.

The duplex scan technique (real time scanner combined with pulsed Doppler equipment) allows precise localisation of a defined volume within the superior mesenteric artery from which the Doppler flow signal is sampled. This study describes the method of measuring blood flow in the superior mesenteric arteries of premature infants. 'Normal' values and characteristic changes in flow patterns of 22 preterm neonates were correlated with enteral feeding and the amount of milk given; peak velocity, mean velocity (integral of the maximum velocities over cardiac cycle), and pulsatility index were analysed.

Patients and methods

The data obtained from 22 preterm infants (gestational age range 25–36 weeks, weight at examination range 600–2850 g, and postnatal age range 1 hour–30 days) were evaluated. At ultrasound examination the general condition of the infants was good; oxygen and carbon dioxide tensions and blood pressures were normal, and their abdomens were free of gas. There were no signs or symptoms of necrotising enterocolitis. Eight patients with hyaline membrane disease required mechanical ventilation. Doppler investigations were carried out in these infants during the first days of life only, to minimise the influence of severe patent ductus arteriosus. Because the infants tolerated the procedure well, sedatives were unnecessary. All preterm infants were fed predefined, gradually increasing, quantities of milk through a gastric tube at intervals of 2, 2·5, or 3 hours—that is, 12, 10, or eight feeds a day. The milk was injected by syringe into the feeding tube over a one to five minute period.

An ATL Ultramark 8 duplex scanner (Scientific Medical Systems), consisting of a real time 7·5 MHz sector scanner and associated 5 MHz pulsed Doppler flowmeter, was used. The signals were analysed using an integral fast Fourier transform processor. The superior mesenteric artery was localised by real time imaging on a horizontal or longitudinal abdominal section, which allowed placement of the sample volume (axial length 1·5–3 mm, lateral dimension 2 mm) at the desired point within the superior mesenteric artery—that is, about 2 to 4 mm distal from where it branches off from the descending aorta (fig 1). In most cases the angle between ultrasound beam and blood flow in the vessel was close to 0 degrees (range, 0–20°); angle correction of the velocities was made automatically. Because of the low inspiratory peak pressures used in the ventilated infants, respiratory movements did not interfere with the measurements. The diaphragmatic movements were comparable with those in normal respiration. The position of the sample volume was optimised by listening to and observing the spectral display, then the recordings were made (usually in the longitudinal plane). The spectrally analysed flow signals of the Doppler shift (digitised every 5 ms) were recorded in real time on a thermoprinter with time on the horizontal axis, Doppler shift frequencies on the vertical axis (velocity), and a grey scale indicating the quantity of blood flowing at that particular velocity (fig 2). After recording the optimised Doppler signals for 10 to 20 seconds, five homogeneous cardiac cycles were measured. Investigations were carried out in fasting infants, preprandially, and in conjunction with milk intake (15, 45, and 90 minutes after feeding). The influence of the quantity of milk was verified in the following three groups set up according to daily milk intake: group A—fasting preterms, group B—<50 ml milk/kg body weight a day, and group C—50 ml or more of milk/kg body weight a day. Statistical analysis was by the Kruskal-Wallis and the Wilcoxon rank sum tests. A p value of $<0{\cdot}05$ was taken as significant.

Results

Sixteen of the 22 preterm infants were examined once, five twice, and one three times with varying quantities of food. A total of 29 Doppler recordings were made for the three groups. Within each group (A, B, or C) each baby had Doppler measurements made once.

Individual fasting or before feeding values for peak velocity, mean velocity, and pulsatility index in the three groups are shown in fig 3, and the mean (SD) result for each group is shown in the table. Peak velocity and mean velocity increased as the quantity of milk increased; the pulsatility index decreased analogously.

The changes of the flow measurements after feeding are shown in fig 4. The data are based on 60 Doppler recordings of 16 preterm infants for whom, in addition to before feeding basal values, had 15 and 45 minute (n=4) or 15, 45, and 90 minute (n=12) values also recorded after feeding. Each curve represents a different patient. These recordings include patients with varying food intake (groups B and C), and therefore mean values and

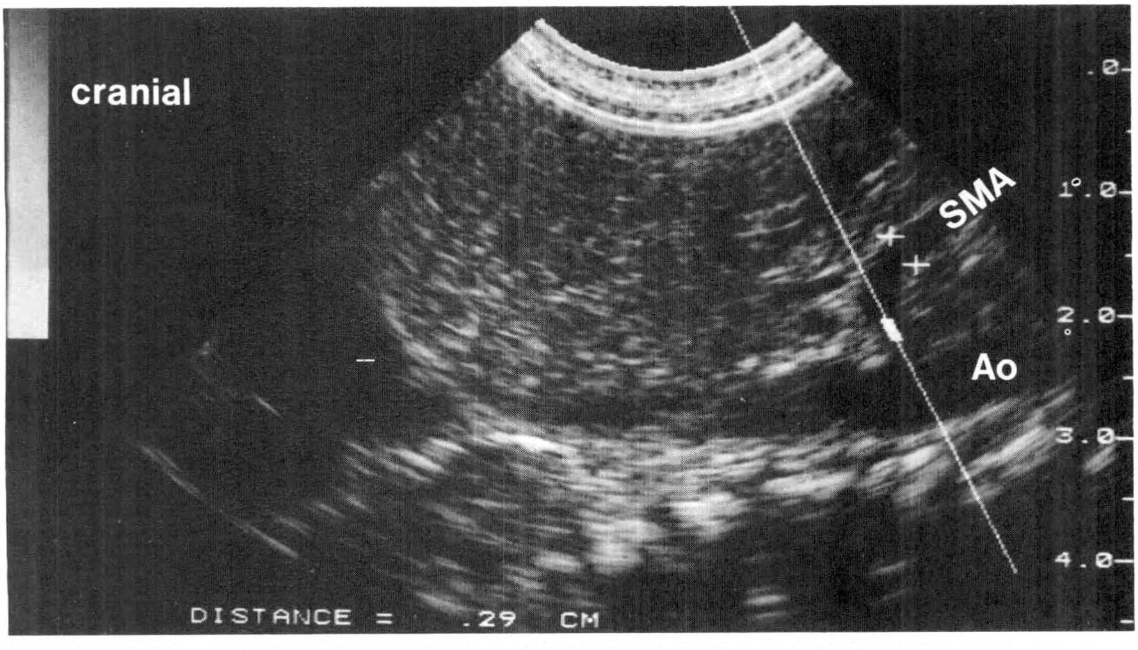

Fig 1 *Sample volume within superior mesenteric artery (SMA). Diameter is 2·9 mm (marked by X). Ao=Aorta.*

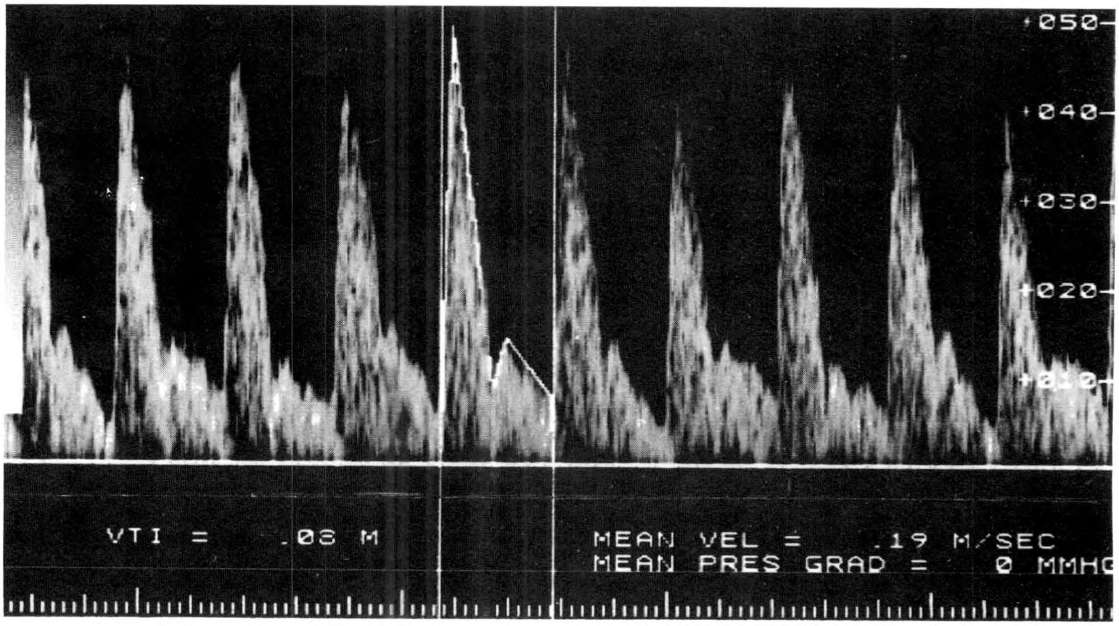

Fig 2 *Flow signal of superior mesenteric artery. Preprandial velocity is shown on the vertical axis (40–45 cm/s). Area below white outline represents mean velocity (integral of maximum velocities over cardiac cycle), which is 19 cm/s.*

standard deviations after feeding were not calculated because their wide distributions would have been misleading. The postprandial increase of flow rate was analysed by the Wilcoxon rank sum test. The curves show a steep, significant rise in peak velocity and mean velocity 15 minutes after feeding, whereas pulsatility index fell. Peak velocity rose significantly again between the 15 and 45 minute values. The flow rate peaked at 45 minutes the minimum interval between feedings was two hours.

Discussion

Blood flow disturbances are an integral part of the aetiology of neonatal abdominal disorders, especially necrotising enterocolitis.[6-8] Measurements of intestinal circulation in adults[2 3] by pulsed Doppler ultrasound of the superior mesenteric artery showed significant changes in flow measurements in association with food intake. Qamar *et al*[9] reported that the response of superior mesenteric artery blood flow even differed depending on the caloric content of the meal (carbohydrate, fat, protein).

Improvement of sonographic techniques with high resolution scan heads (7·5 MHz) has made imaging of minute vessels like the superior mesenteric artery in newborns and very low birthweight infants possible. The combined Doppler method has considerably widened our knowledge of neonatal intestinal circulation. Leidig[4] investigated flow patterns and normal values in healthy newborns. The present study shows that preterm infants are able to regulate blood flow in the superior mesenteric artery in association with milk intake; in addition, baseline values are reported.

Evaluating the correlation between intestinal perfusion and postpartum age is difficult, because our patients received milk orally 12 to 24 hours after birth. The loading effect of milk on the intestine, therefore, cannot be differentiated from possible changes resulting from increasing postnatal age. The pure time dependent effect can be shown only after several days of fasting. Given that with the regular daily increase in milk intake the postnatal age is associated with the quantity of milk, testing the effect of varying quantities of milk also indirectly

Table *Mean (SD) Doppler parameters in the three groups*

	Group A (n=7)	*Group B (n=12)*	*Group C (n=10)*
Peak velocity (cm/sec)	34 (6)	46 (10)	56 (6)
Mean velocity (cm/sec)	10 (2)	15 (4)	22 (5)
Pulsatility index	0·94 (0·06)	0·90 (0·09)	0·86 (0·09)

examined the time dependency of blood flow in the superior mesenteric artery. Postnatal ages in group A, B, and C were as follows: group A—1–18 hours, group B—1–9 days, and group C—10–30 days.

The significant increase in peak velocity and mean velocity and the declining pulsatility index in fig 3 shows that the quantity of milk serves as an index of superior mesenteric artery perfusion. In contrast to Daneman *et al*[5] (who found no significant consistent decrease in pulsatility index after enteral feeding in six premature infants) the preterm infants examined and analysed in this study, showed a definite change in all flow measurements after meals—that is, decreasing pulsatility index, increasing peak velocity, and increasing mean velocity (fig 4). Because the pulsatility of the recorded Doppler wave form is associated with the vascular impedance downstream to the point of measurement,[10] intestinal vascular resistance after feeding reaches its nadir between 15 and 45 minutes after feeding.

The pulsatility in Doppler recordings, especially in premature infants receiving assisted ventilation, depends primarily on the severity of patent ductus arteriosus. Mean flow velocity values are lower because of the reduced diastolic flow, which in some cases is even retrograde with pulsatility index values of more than 1·00. The three pulsatility index values over 1·00 in fig 3 were measured in ventilated infants and should be attributed to the effect of a mild patent ductus arteriosus. With the exception of these raised pulsatility index values, the flow measurements did not differ between ventilated infants and those with spontaneous respiration (critically ill infants with severe patent ductus arteriosus were excluded). A reduction of intestinal circulation during feeding is to be expected in critically ill ventilated babies, particularly those with demonstrable holodiastolic backflow into the superior mesenteric artery. In such cases, a delay in enteral feeding and gradual increase of food intake (maximum daily increase, 5 g food/kg body weight) are indicated to prevent such gastrointestinal complications as extensive bowel gas, vomiting, and malnutrition. Further investigations are necessary to establish the efficacy of individual superior mesenteric artery flow measurements for the evaluation

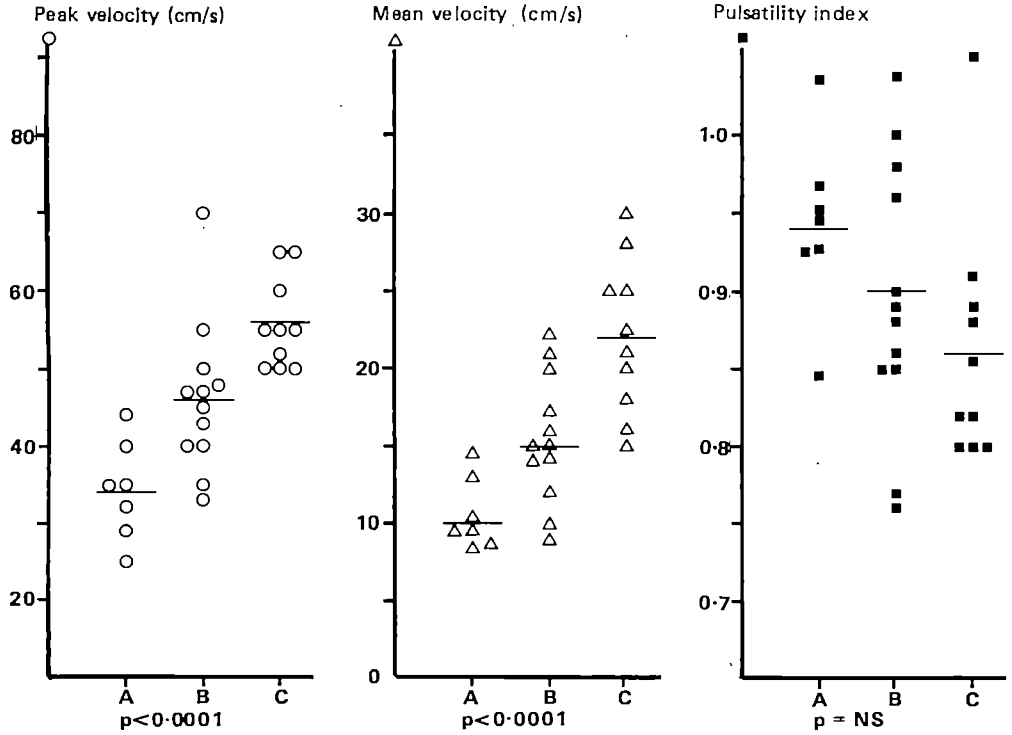

Fig 3 *Peak velocity, mean velocity, and pulsatility index in babies who had not been fed (A) or who had received <50 ml milk/kg body weight (B) or ≥50 ml milk/kg body weight (C). Measurements in groups B and C were made immediately before a feed. Horizontal lines indicate mean of individual values.*

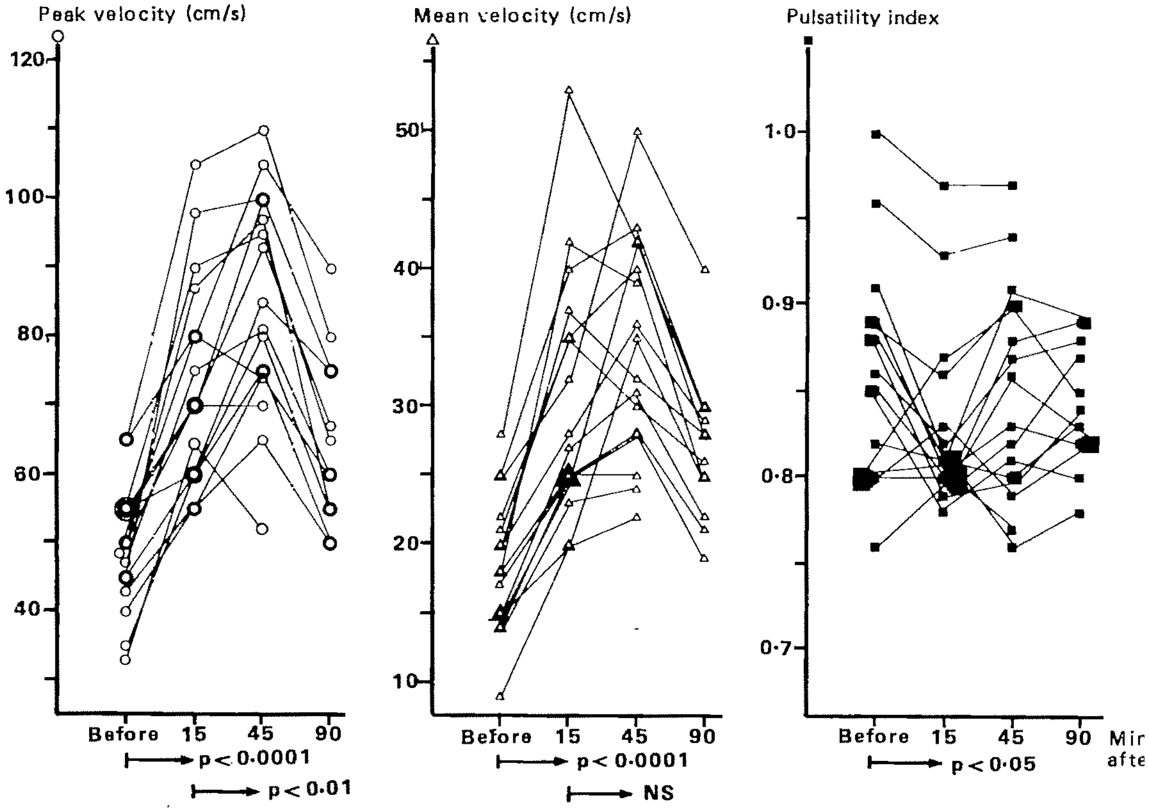

Fig 4 *Response of peak velocity, mean velocity, and pulsatility index to feeding (n=16). Large symbols represent identical values.*

and quantification of reduced intestinal perfusion in newborns and premature infants with gastrointestinal disease.

References

[1] Tepperman BL, Jacobsson ED. Measurement of gastrointestinal blood flow. *Ann Rev Physiol* 1982;**44**:71–82.

[2] Taylor KJW, Burns PN, Woodcock JP, Wells PNT. Blood flow in deep abdominal and pelvic vessels: ultrasonic pulsed-Doppler analysis. *Radiology* 1985;**154**:487–93.

[3] Qamar MI, Read AE, Skidmore R, Evans JM, Wells PNT. Transcutaneous Doppler ultrasound measurement of superior mesenteric artery blood flow in man. *Gut* 1986;**27**:100–5.

[4] Leidig E. Pulsed Doppler ultrasound blood flow measurements in the superior mesenteric artery of the newborn. *Pediatr Radiol* 1988 (in press).

[5] Daneman A, Babyn P, Garcia P, Stringer DA, Hellman J. Doppler sonography of intraabdominal vessels in premature infants. *Pediatr Radiol* 1987;**17**:342 (A).

[6] Obladen M. Necrotizing enterocolitis. *Monatsschr Kinderheilkd* 1986;**134**:515–9.

[7] Hackett GA, Campbell S, Gamsu H, Cohen-Overbeek T, Pearce JMF. Doppler studies in the growth retarded fetus and prediction of neonatal necrotizing enterocolitis, haemorrhage, and neonatal morbidity. *Br Med J* 1987;**294**:13–6.

[8] Deeg KH, Gerstner R, Bundscherer F, Harai G, Singer H, Gutheil H. Doppler sonographic detection of reduced flow velocities in the truncus coeliacus in infants with patent ductus arteriosus Botalli in comparison to a healthy control group. *Monatsschr Kinderheilkd* 1987;**135**:24–9.

[9] Qamar MI, Read AE, Mountford R, Skidmore R, Wells PNT. Effect of carbohydrate, fat and protein on superior mesenteric artery blood flow in man. *Gut* 1984;**25**:1154(A).

[10] Evans DH, Barrie WW, Asher MJ, Bentley S, Bell PR. The relationship between ultrasonic pulsatility index and proximal arterial stenosis in a canine model. *Circ Res* 1980;**46**:470–5.

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Megakaryocytopoiesis in the human fetus

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SUMMARY After immunohistochemical staining the size and maturational stage of fetal megakaryocytes from 20 human fetuses of 12 to 21 weeks' gestation were compared with those from normal adults. The mean diameter of fetal megakaryocytes was 14·0 microns when stained with antiglycoprotein IIb (Tab) and 15·2 microns when stained with antifactor VIII, which was significantly smaller than adult megakaryocytes, which were 18·4 microns when stained with Tab and 20·6 microns when stained with factor VIII. The proportion of immature (stage II) cells was higher—and the proportion of mature (stage IV) cells was lower—in the fetal tissue than in the adult.

The smaller size and shift to a less mature population indicated that there were differences at the non-mitotic phase of the development of megakaryocytes in the fetus. Such differences were probably associated with quantitative and qualitative platelet abnormalities in newborn infants. Understanding the physiology and regulation of megakaryocytopoiesis in fetuses and newborn infants will be invaluable in determining the pathophysiology of platelet dysfunction in the newborn.

Platelets in newborn infants are quantitatively and qualitatively different from those in older children and adults[1]; The lowest normal platelet count may be as low as $100\times10^9/l$ in the newborn. Newborn infants are also vulnerable to thrombocytopenia, there being an incidence of 10 to 60% in ill newborn infants, and thrombocytopenic babies are at increased risk of intracranial haemorrhage and death.[1-3] Functional abnormalities in platelets from neonates include decreased adhesion to glass fibres, low platelet factor III activity, and hypoaggregability to adenosine diphosphate, epinephrine, thrombin, and collagen.[1 4] There is also evidence of abnormal clot retraction and abnormal release of platelet factor IV in the newborn.[5 6]

The metabolic capacity of the platelet—and perhaps its adaptability—is established in the precursor cell, the megakaryocyte,[7] and it is likely that fetal megakaryocytes differ from adult megakaryocytes. Our hypothesis is that immature megakaryocytes produce platelets that differ in number and function from platelets produced by mature megakaryocytes. In this study the size and maturational stage distribution of fetal megakaryocytes were examined using an immunohistochemical staining procedure and then compared with normal adult marrow; there was morphological evidence of reduced megakaryocyte maturity in the fetus.

Material and methods

SPECIMENS

Human fetal tissue was obtained from 18 normal and two anencephalic aborted fetuses of 12 to 21 weeks' gestation after vacuum aspiration. Biopsy specimens of bone marrow were obtained from the femurs, and specimens from liver and spleen were collected when possible. Adult bone marrow trephine biopsy specimens were collected from the iliac crest of five normal volunteers after obtaining informed consent. All studies were approved by the Institutional Review Board.

Fixation and embedding were as described by Beckstead.[8] Biopsy specimens were fixed in paraformaldehyde, serially dehydrated in acetone, then infiltrated and embedded in glycol methacrylate (JB4, Polysciences Inc). Sections were cut 3·0 microns thick with glass knives and mounted on glass slides precoated with 0·1% poly-L-lysine.

IMMUNOHISTOCHEMICAL STAIN

The staining procedure was modified from the procedure described by Beckstead *et al.*[8] Biopsy sections mounted on glass slides were incubated in 0·25% trypsin, then in 3% normal sheep or goat serum using the same species as used for the secondary antibody. The sections were incubated in primary antibody, mouse monoclonal antiplatelet glycoprotein IIb (Tab), or rabbit polyclonal antihuman factor VIII (Calbiochem-Behring Corporation) for 2 to 2½ hours at 37°C, and then in secondary antibody, biotinylated sheep antimouse IgG, or biotinylated goat antirabbit IgG (Organon Teknika-Cappel) for one hour at 37°C. Endogenous peroxidase was inhibited with 0·3% hydrogen peroxide in 30% methanol for three minutes, and then in avidin peroxidase conjugate diluted 1/80 at room temperature for one hour. The chromogenic substrate 3, 3-diaminobenzidene (DAB) 0·05% was added, and then a solution of 0·05% DAB, 0·1% hydrogen peroxide, imidazole, and sodium azide. Further colour development was achieved with 0·5% copper sulphate, and counterstained with haematoxylin before bluing in Scott's water.

Most sections of marrow and all specimens of liver and spleen were also stained with Wright-Giemsa stain or haematoxylin and eosin for comparison.

MEASUREMENT OF THE SIZE OF MEGAKARYOCYTES

Megakaryocytes were identified by specific brown DAB staining. The largest axis of every megakaryocyte was measured to the nearest micron with a calibrated eyepiece reticle at a magnification of 400. Megakaryocyte cytoplasmic fragments, and those without nuclei, were not measured.

ASSESSMENT OF THE MATURATIONAL STAGE OF MEGAKARYOCYTES

The approximate maturational stage of each megakaryocyte was assessed as stage I-IV, guided by criteria elaborated by Levine *et al.*[9] Stage I cells were comparatively small with a high nuclear:cytoplasmic ratio and a single or bilobed nucleus. Stage II cells were larger with more complex nuclear lobulation than stage I cells; stage II cells also had a comparatively high nuclear:cytoplasmic ratio although it was less than in stage I cells. Stage III cells were larger with abundant cytoplasm and complex multilobulated nuclei with separated nuclear lobes. Stage IV cells were large with abundant cytoplasm and compact multilobulated nuclei.

STATISTICAL ANALYSIS

Megakaryocytes may be treated as spheres with minimum error, even when they look ellipsoidal.[10 11] Harker has shown that the mean of the diameter measurements of biopsy sections gives a value that is 87% of the true diameter; that value increases to 93% of the true diameter if only cells containing nuclei are counted.[11] Measurements were therefore divided by 0·93 to represent the actual megakaryocyte diameter.

Because large cells are cut more times and therefore represented in more sections than are smaller cells, the number of cells observed must be corrected for multiple counting. The data were grouped by size and pooled. The number (n) of cells at each size was divided by the correction factor (d/t+1), where d equals the corresponding diameter and t equals the section thickness (3 microns)[10 11]; n corrected = n observed in section/(d/t+1).

The mean and standard deviation were calculated as for grouped data. Mean diameters were compared by the two sample *t* test for samples with equal variance or with unequal variance (equality of variances was assessed by the F test). Trends in size and stage distribution *v* fetal age were evaluated using regression analysis. Comparison of proportions of cells at various maturational stages were made with 2×2 contingency tables and χ^2 with Yates's correction.

Results

Twenty specimens of fetal marrow were stained with Tab antibody, and 15 with factor VIII antibody, both groups including two anencephalic fetuses, and spanning the gestational age group 12 to 21 weeks. Specimens of liver were obtained from 11 fetuses and of spleen from six fetuses. Five biopsy specimens of normal adult bone marrow were examined with Tab antibody and four with factor VIII antibody.

All megakaryocytes identified by immunostaining were counted and assessed. The median was 107 megakaryocytes/section, range 11–195. Femurs from the two youngest fetuses, of 12 and 13 weeks' gestation, had primarily loose mesenchymal tissue with immature cartilage and bone and only small sparse foci of haematopoiesis. In both fetal and adult specimens the distribution and size of the megakaryocytes varied through the specimen, with clusters of immature cells separated from clusters of mature cells. Almost all the megakaryocytes had diffuse cytoplasmic staining with increased intensity at the cytoplasmic membranes. Megakaryocytes in the adult specimens generally stained darker than fetal megakaryocytes. Although the staining procedure included a step to block endogenous peroxidase, slight non-specific staining in erythroid precursors occasionally occurred; the characteristic nuclear features of erythroid precursors and megakaryocytes were of use in differentiating between these small cells. Most osteoclasts also stained weakly with the immunohistochemical procedure, but the unique nuclear features, smooth outline, and proximity to bone identified them.

Megakaryocytes from the anencephalic fetuses were of the same size and had the same stage distribution as those from normal fetuses.

SIZE

The mean (SD) diameter of fetal megakaryocytes identified with Tab antibody was 15·2 (1·4) microns, significantly smaller than the mean diameter of adult megakaryocytes, 20·6 (4·1) microns (p=0·04). The mean diameter of fetal megakaryocytes increased from 12·2 microns at stage I to 22·6 microns at stage IV. When each stage in the group stained with Tab was examined individually, at stages II, III, and IV, the fetal megakaryocytes were significantly smaller than adult megakaryocytes (table 1).

Similarly, the fetal megakaryocytes stained with factor VIII were smaller than the adult, mean diameters being 14·0 (1·2) and 18·4 (2·1) microns, respectively (p<0·0001). At all stages the fetal megakaryocytes were smaller than the adult megakaryocytes (table 1).

The size of the megakaryocytes did not vary significantly over the gestational age range of 12–21 weeks.

STAGE

In both the fetal and adult specimens the largest proportion of megakaryocytes was assessed as immature or stage I (table 2). The fetal specimens had a higher proportion of stage II immature megakaryocytes than the adult specimens, 28% and 22% (p=0·004) for those stained with Tab and 30% and 21% (p<0·001) for those stained with factor VIII. There were significantly fewer stage IV mature cells in the fetal specimens than in the adult, 8% and 16% in the group stained with Tab and 9% and 19% in those stained with factor VIII (p<0·001). There was no significant association between gestational age and stage distribution for the gestational range examined.

Liver and spleen

Eleven liver biopsy specimens of 12–21 weeks' gestation and six biopsy specimens of spleen of 16–21 weeks' gestation showed nests of hematopoiesis with a decline in the hematopoietic population density with advancing gestation. Few megakaryocytes were present, their diameter and morphological appearances being similar to those in fetal marrow; few mature megakaryocytes were found. The immature megakaryocytes were most easily located by immunostaining and the mature megakaryocytes by Wright-Giemsa staining.

Discussion

This study shows that during the second trimester of gestation the fetal megakaryocytes are smaller than adult megakaryocytes. The mean adult megakaryocyte (SD) diameter in this study was similar to that found by Harker, being 20·8 (3·3) microns.[11] There is little information about megakaryocyte size in the fetus. Izumi *et al* examined the size of megakaryo-

Table 1 *Mean (SD) diameter (microns) of megakaryocytes in biopsy specimens corrected for sampling method*

	Stained with antiglycoprotein IIb			*Stained with factor VIII*		
	Fetal	*Adult*	*p Value**	*Fetal*	*Adult*	*p Value**
Stage I	12·2 (1·2)	15·2 (3·9)	0·2	11·0 (0·9)	12·4 (0·9)	0·01
Stage II	15·8 (1·1)	22·4 (2·4)	0·003	14·2 (1·0)	19·1 (2·9)	0·04
Stage III	20·7 (4·9)	29·2 (1·3)	<0·001	20·0 (1·8)	26·1 (3·0)	<0·001
Stage IV	22·6 (5·4)	29·3 (2·4)	<0·001	19·9 (5·9)	26·4 (1·5)	<0·001
Total	15·2 (1·4)	20·6 (4·1)	0·04	14·0 (1·2)	18·4 (2·1)	<0·001

*Two sample *t* test.

Table 2 *Percentage distribution of megakaryocytes by age*

	Stained with antiglycoprotein IIb			*Stained with factor VIII*		
	Fetal	*Adult*	*p Value*	*Fetal*	*Adult*	*p Value*
Stage I	50	49	0·7	48	46	0·5
Stage II	28	22	0·004	30	22	<0·001
Stage III	14	13	0·3	14	13	1·0
Stage IV	8	16	<0·001	8	19	<0·001
Total	100	100	<0·001*	100	100	<0·001*

*χ^2 test, 4×2 contingency table.

cytes over a range of ages and found that a 5 month old fetus had smaller cells than a 7 month old fetus; in turn, fetal megakaryocytes were smaller than infant, and infant smaller than child and adult megakaryocytes. The megakaryocytes reached adult size at about 1 year of age.[12]

The high proportion of stage I immature megakaryocytes was an unexpected finding with several possible explanations. Promegakaryoblasts are small and generally not morphologically distinguishable from lymphocytes or other blast cells, so specific staining techniques are needed to identify them. Previous reports may have used enrichment procedures which exclude the small, higher density cells, and diminish the immature population. In biopsy sections visual impression makes large, mature megakaryocytes seem predominant, and without correction for multiple counting the larger cells are disproportionately represented. With the DAB staining procedure used in this study endogenous peroxidase in erythroblasts may produce artifactual staining, which causes them to be counted as stage I megakaryocytes. Even if such an artifact exists, comparison of fetal with adult is still useful. When only stages II, III, and IV are evaluated there is a clear difference in maturational distribution between fetus and adult, with the balance being towards less mature forms in the fetus.

Stage distribution of megakaryocytes has not been widely reported. Rabellino *et al* studied marrow from human ribs and found the ratio of small cells to mature megakaryocytes was about 1:2; the small cells represented the early megakaryocytic differentiation compartment.[13] Levine and Fedorko found that harvested guinea pig marrow cells in suspension contained an average of 25% immature, 50% intermediate, and 20% mature megakaryocytes; a concentrated population consisted of 21% immature, 62% intermediate, and 17% mature megakaryocytes.[14]

Evaluation of maturational stage in biopsy sections presented the following consideration: assessment of stage relies heavily on nuclear characteristics but only a random section of nucleus is represented on the slide, which is a potential source of error. The relative associations, however (stage I less mature than stage II, II less than III, and so on) would still be valid when applied to a large number of cells. For example, a small cell filled with a single lobed nucleus and a thin rim of cytoplasm is less mature than a cell with two nuclear lobes that only partially fill the cell.

Mature or advanced stage megakaryocytes are generally larger and have a higher DNA content, or ploidy. The quantity of DNA seems to be associated with the qualitative characteristics of platelets, cytoplasmic volume, and quantitative platelet production.[9 15–17]

The findings from this study indicate that fetal megakaryocytes are less mature than adult megakaryocytes. The morphological findings of smaller size and shift to a less mature population in the fetus are likely to be associated with quantitative and qualitative abnormalities in platelet of the newborn.

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References

[1] de Alarcon PA. Thrombopoiesis in the fetus and newborn. In: Stockman JA III, Pochedly C, eds. *Developmental and neonatal hematology*. New York: Raven Press, 1988:103–30.

[2] Andrew M, Castle V, Saigal S, Carter C, Kelton JG. Clinical impact of neonatal thrombocytopenia. *J Pediatr* 1987;**110:** 457–64.

[3] Castle V, Coates G, Kelton JG, Andrew M. ^{111}In-oxine platelet survivals in thrombocytopenic infants. *Blood* 1987;**70:**652–6.

[4] Hrodek O. L'agregation des plaquettes chez le nouveau-né. *Nauv Rev Fr Hematol* 1969;**9:**569–73.

[5] Mull MM, Hathaway WE. Altered platelet function in newborns. *Pediatr Res* 1970;**4:**229–37.

[6] Whaun JM, Smith GR, Sochor VA. Effect of prenatal drug administration on maternal and neonatal platelet aggregation and PF release. *Hemostasis* 1980;**4:**226–37.

[7] Ebbe S. Biology of megakaryocytes. *Prog Hemost Thromb* 1976;**3:**211–29.

[8] Beckstead JH, Stenberg PE, McEver RP, Shuman MA, Bainton DF. Immunohistochemical localization of membrane and alpha-granule proteins in human megakaryocytes: application to plastic-embedded bone marrow biopsy specimens. *Blood* 1986; **67:**285–93.

[9] Levine RF, Hazzard KC, Lamberg JD. The significance of megakaryocyte size. *Blood* 1982;**60:**1122–31.

[10] Cullen WC, McDonald TP. Comparison of stereologic techniques for the quantification of megakaryocyte size and number. *Exp Hematol* 1986;**14:**782–8.

[11] Harker LA. Megakaryocyte quantitation. *J Clin Invest* 1968;**47:** 252–7.

[12] Izumi T, Kawakami M, Enzan H, Ohkita T. The size of megakaryocytes in human fetal, infantile and adult hematopoiesis. *Hiroshima J Med Sci* 1983;**32:**257–60.

[13] Rabellino EM, Levene RB, Leung LLK, Nachman RL. Human megakaryocytes. II. Expression of platelet proteins in early marrow megakaryocytes. *J Exp Med* 1981;**154:**88–100.

[14] Levine RF, Fedorko ME. Isolation of intact megakaryocytes from guinea pig femoral marrow. *J Cell Biol* 1976;**69:**159–72.

[15] Harker LA. Control of platelet production. *Annu Rev Med* 1974;**25:**383–400.

[16] Bessman JD. The relation of megakaryocyte ploidy to platelet volume. *Am J Hematol* 1984;**16:**161–70.

[17] Thompson CB, Jakubowski JA, Quinn PG, Deykin D, Valeri CR. Platelet size and age determine platelet function independently. *Blood* 1984;**63:**1372–5.

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Osteopenia in extremely low birthweight infants

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SUMMARY Fifteen extremely low birthweight (ELBW) white infants (those weighing 1000 g or less) were observed at birth, within eight weeks of birth and near 40 weeks postconception. On the second and third occasions, weight, crown-heel length, and bone mineral content of their forearms were measured. Fifteen infants born at full term on whom similar measurements were made soon after birth acted as controls. Between 32 and 39 weeks the median weight of ELBW infants increased from 970 g to 1850 g and crown-heel length from 35·7 cm to 41·0 cm. There was no evidence, however, of bone mineral accretion in the measurement region; initial and final median measurements of bone mineral content were 76 mg/cm and 86 mg/cm, the median individual difference being only 4 mg/cm with an interquartile range of 25 mg/cm. Median weight, crown-heel length, and bone mineral content of the control group were 3270 g, 50·6 cm, and 196 mg/cm, respectively. Compared with the controls, ELBW infants at 39 weeks were a median (interquartile range) of 1420 (525) g lighter, 9·9 (3·9) cm shorter, and had a bone mineral content deficit of 108 (32) mg/cm.

In terms of weight and crown-heel length ELBW infants at 39 weeks were comparable with infants born and observed at 32 weeks' gestation; compared with these infants the bone mineral content deficit in the ELBW group was about 33%.

It has been shown that in neonates the bone mineral content of the radius and ulna can be measured precisely and accurately by single photon absorptiometry.[1–5] Using that technique we have shown that there is a substantial deficit in bone mineral content in most preterm infants when they reach a postconceptional age of 40 weeks compared with infants born at full term who were observed at birth.[2]

Such a deficit may be the result of reduced density of mineralisation caused by a low rate of mineral accretion on existing organic matrix, or by a reduction in bone size caused by impaired skeletal growth, or a combination of the two. The relative contribution of each factor to undermineralisation in preterm infants is unknown, but has important implications for the assessment and treatment of osteopenia of prematurity. There is evidence, for example, that reduced rates of mineral accretion can be improved by mineral supplementation,[3] and that radiographically detectable undermineralisation can be ameliorated by vitamin D supplementation.[6] In contrast, reduced bone growth tends to be associated with reductions in general measurements of growth such as the rates of increase of body weight and crown-heel length, which may reflect inadequate nutrition in general.

To assess the relative contributions of a reduction in the density of mineralisation and bone size to the known bone mineral content deficit in preterm infants at 40 weeks, we studied a series of extremely low birthweight (ELBW) infants (those weighing 1000 g or less) who as a group were expected to have a more severe degree of undermineralisation than larger infants.[7]

Patients and methods

Parental consent was obtained to carry out the investigations in every case and the study had the approval of the local hospital ethics committee.

PRETERM ELBW INFANTS

Fifteen preterm white infants (eight of whom were boys) of birth weight 1000 g or less were recruited over a three year period. Gestational ages were calculated from maternal menstrual history and where possible were confirmed by either external examination[8] or by ophthalmoscopic examination of the lens (table 1).[9] Gestational ages ranged from 25 to 30 weeks. Birth weights ranged from 650 to 1000 g, and were appropriate for gestational age in all but two cases, cases 2 and 5.

In all cases the bone mineral content (mg/cm) was measured within eight weeks of birth at the middle of the right forearm by photon absorptiometry.[1 2] The amount of radiation was small, the skin entry dose being 0·03 mGy/investigation. Postconceptional ages at that time ranged from 29 to 35 weeks; weight (g) and, except in one case, crown-heel length (cm) were measured just before the scan. Between 37 and 42 weeks after conception, the ELBW infants' weights, crown-heel lengths and bone mineral contents were measured again (tables 2 and 3).

CONTROLS GROUP BORN AT FULL TERM

Fifteen white infants born at full term were identified from our existing database to serve as controls. Each one had had measurements of weight, crown-heel length, and bone mineral content carried out within one week of birth and could be matched with an ELBW infant, the matching variable being post-

Table 1 *Sex, gestational age at birth, and weight of the matched pairs of ELBW and control infants born at full term*

Pair No	*ELBW infants*			*Infants born at full term*		
	Sex	*Gestation (weeks)*	*Weight (g)*	*Sex*	*Gestation (weeks)*	*Weight (g)*
1	Female	27	650	Female	38	3440
2	Male	29	725	Female	38	3470
3	Male	27	740	Male	40	3070
4	Female	27	780	Female	38	2900
5	Female	30	800	Female	38	2980
6	Female	26	840	Female	41	4440
7	Male	25	925	Male	39	3680
8	Female	26	950	Female	38	3430
9	Male	27	950	Male	39	4300
10	Female	26	960	Female	40	3930
11	Male	28	1000	Male	39	3840
12	Male	28	1000	Male	40	4160
13	Male	27	1000	Male	38	3420
14	Female	28	1000	Female	37	2880
15	Male	29	1000	Male	37	2840
Median (interquartile range)		27 (2)	950 (220)		38 (2)	3430 (950)

Table 2 *Postconceptional age, weight, crown-heel length, and bone mineral content of the ELBW infants on the first occasion that absorptiometry was carried out*

Case No	*Postconceptional age (weeks)*	*Weight (g)*	*Crown-heel length (cm)*	*Bone mineral content (mg/cm)*
1	30·43	700	33·1	75·58
2	30·29	675	33·4	58·81
3	34·71	970	37·0	72·01
4	34·00	1025	35·0	89·99
5	33·43	1050	36·2	60·61
6	30·14	800	35·6	96·54
7	29·00	910	35·2	72·11
8	31·86	950	—	72·21
9	30·57	975	38·2	75·27
10	29·29	825	35·3	71·62
11	30·71	1000	39·5	98·69
12	31·43	955	35·6	79·09
13	35·00	1100	35·8	106·87
14	31·14	1250	38·9	79·43
15	31·29	1000	37·1	89·78
Median (interquartile range)	31·14 (3·14)	970 (200)	35·7 (1·9)	75·58 (17·98)

Table 3 *Postconceptional age, weight, crown-heel length, and bone mineral content of the ELBW infants on the second occasion that absorptiometry was performed, and of the control infants born at full term on the first occasion that absorptiometry was performed (soon after birth)*

Pair No	*ELBW infants*				*Infants born at full term*			
	Postconceptional age (weeks)	*Weight (g)*	*Crown-heel length (cm)*	*Bone mineral content (mg/cm)*	*Postconceptional age (weeks)*	*Weight (g)*	*Crown-heel length (cm)*	*Bone mineral content (mg/cm)*
1	38·14	1850	41·0	64·95	38·43	3370	48·6	143·06
2	38·14	1500	39·2	65·04	38·71	3270	48·6	195·66
3	40·57	1620	39·5	89·18	40·57	2320	50·6	180·39
4	38·00	1440	39·8	65·95	38·43	2300	49·7	208·36
5	37·43	1600	39·6	68·72	38·14	2380	51·0	191·77
6	41·29	2520	46·0	79·86	41·14	4440	60·0	208·86
7	39·14	2395	43·5	91·61	39·14	3580	51·0	205·43
8	38·71	2170	44·3	86·26	38·71	3250	50·6	204·13
9	39·00	1850	43·1	68·80	39·00	4300	54·3	182·45
10	40·86	3050	47·7	100·66	40·86	3830	54·0	173·76
11	39·86	1900	40·9	102·25	39·86	3710	53·0	197·34
12	40·86	1980	42·7	90·35	40·86	4060	54·2	198·82
13	38·71	1400	39·1	109·23	39·00	3030	49·0	217·50
14	37·14	1700	43·5	60·88	37·14	2880	49·7	127·32
15	37·00	1350	39·7	92·06	37·29	2770	48·8	183·90
Median (interquartile range)	38·71 (2·57)	1850 (670)	41·0 (3·9)	86·26 (26·11)	39·00 (2·14)	3270 (910)	50·6 (5·0)	195·66 (25·04)

conceptional age at the time the scan was performed; for the ELBW infant, this was the age at the second scan. Ages were matched to within one week and with one exception the pairs were matched for sex (tables 1 and 3).

STATISTICAL ANALYSIS

Non-parametric tests were used throughout. Null hypotheses that paired groups had common medians were tested using the Wilcoxon signed rank test. Rank correlation coefficients were compared using Spearman's test. Because we have shown that intrauterine growth curves for weight, crown-heel length, and bone mineral content are exponential,[10] logarithms of the measurements were used to examine correlations between rates of change.

Results

ELBW INFANTS

In the interval between birth and the first scan (median (interquartile) 3·4 (2·7) weeks), the median weight gain (g) in the ELBW infants was zero (interquartile range 270 g). Thus at the time of the first scan the distribution of weights in that group was similar to the distribution of weights at birth.

The second scans were performed at a median postconceptional age of 38·7 weeks, the median (interquartile) time interval between the first and second scans being 7·7 (3·7) weeks. During that time the median (interquartile) weight increase was 875 (770) g and the median crown-heel length increase was 4·85 (4·60) cm; both increases were significant ($p<0·01$) and individual rates of weight and crown-heel increase were significantly associated ($r=0·56$, $p<0·05$; logarithmic data). Comparing the median increases with the median values at the time of the first scan, weight nearly doubled, and crown-heel length increased by 15%. In contrast, the median (interquartile) change in bone mineral content between the two scans was only 3·56 (24·68) mg/cm, and was not significantly different from zero. There was no evidence in the data to suggest that the ELBW infants with the higher gestational ages at birth had mineralised their bones more rapidly than the remainder.

DIFFERENCES AT 39 WEEKS BETWEEN ELBW INFANTS AND THOSE BORN AT FULL TERM

The median (interquartile) values of postconceptional age at the time of second scan in the ELBW group and the time of the first scan in the control group were 38·7 (2·6) weeks and 39·0 (2·1) weeks, respectively.

The ELBW infants were significantly lighter than those born at full term ($p<0·01$), the median difference between the pairs being 1420 (525) g. In the group born at full term the median weight was

3270 g; the weight deficit in the ELBW group was therefore about 40%. The ELBW infants were also significantly shorter ($p<0·01$), the median crown-heel length difference between the pairs being 9·9 (3·9) cm, about 20% less than the median crown-heel length in those born at full term (50·6 cm). In the ELBW infants, there was significantly less mineral in the region scanned ($p<0·01$), the median difference in bone mineral content between the pairs being 108 (31·84) mg/cm; the median differences in bone mineral content was about 50% of that in those born at full term.

Discussion

Previous radiographic studies have shown disordered bone mineralisation in 75% of ELBW infants.[7] We found severe osteopenia in all cases caused by an almost total failure of mineralisation from birth onwards; at about 39 weeks postconceptional age, every ELBW infant had a bone mineral content value below the lowest control value (127·32 mg/cm).

Because measurements of bone mineral content are influenced by density of mineralisation and by bone size, the osteopenia detected in the ELBW group might have been caused by a mineralisation defect alone, reduced linear growth alone, or a combination of the two. Our data have shown that retardation of growth did occur in a high proportion of the ELBW infants; 14 of the 15 infants at 39 weeks weighed less than the lowest control value (2770 g), and all had crown-heel lengths below the lowest control value (48·6 cm). As a group the ELBW infants at 39 weeks had weights and crown-heel lengths similar to those of newborn infants of gestational ages of 32 weeks or less. Even such a profound reduction in growth rate, however, could not explain the observed mineralisation deficit observed at 39 weeks. The expected mean bone mineral content at birth for a group of infants of 32 weeks' gestation is 123 mg/cm,[10] whereas the mean value in the ELBW infants at 39 weeks was 82·4 mg/cm (median 86·3 mg/cm), 33% less than expected. This deficit indicates what is commonly understood by the term 'metabolic bone disease' and it is this component that is probably shown on standard radiological assessment.

Our results therefore indicate that in ELBW infants osteopenia of prematurity detected by photon absorptiometry at about 40 weeks is caused both by reduced growth and by reduced density of mineralisation. It has previously been suggested that such osteopenia is the result of insufficient dietary intake of mineral, and that dietary mineral supplementation might normalise bone mineral content by the postconceptional age of 40 weeks.[3] It is, however, clear from our results that in the most immature infants growth failure is an important factor in osteopenia at that time. Achievement of normal values of bone mineral content in ELBW infants requires not only successful mineral supplementation, but also successful stimulation of postnatal growth toward the rate achieved in utero.

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References

[1] James JR, Truscott J, Congdon PJ, Horsman A. Measurement of bone mineral content in the human fetus by photon absorptiometry. *Early Hum Dev* 1986;**13**:169–81.

[2] James JR, Congdon PJ, Truscott J, Horsman A, Arthur R. Osteopenia of prematurity. *Arch Dis Child* 1986;**61**:871–6.

[3] Steichen JJ, Tsang RC. Osteopenia of prematurity: the cause and possible treatment. *J Pediatr* 1980;**96**:249–52.

[4] Chan GM, Mileur L, Hansen JW. Effects of increased calcium and phosphorus formulas and human milk on bone mineralisation in preterm infants. *J Pediatr Gastroenterol Nutr* 1986;**5**:444–9.

[5] Greer FR, Lane J, Weiner S, Mazess RB. An accurate and reproducible absorptiometric technique for determining bone mineral content in newborn infants. *Pediatr Res* 1983;**17**:259–62.

[6] Hillman LS, Hollis B, Salmons S, *et al*. Absorption, dosage and effect on mineral homeostasis of 25-hydroxycholecalciferol in premature infants. Comparison with 400 and 800 IU vitamin D_2 supplementation. *J Pediatr* 1985;**106**:981–9.

[7] Lyon AJ, McIntosh N, Wheeler K, Williams JE. Radiological rickets in extremely low birth-weight infants. *Pediatr Radiol* 1987;**17**:56–8.

[8] Parkin JM, Hey EN, Clowes JS. Rapid assessment of gestational age at birth. *Arch Dis Child* 1976;**51**:259–63.

[9] Hittner HM, Hirsch NJ, Rudolph AJ. Assessment of gestational age by examination of the anterior vascular capsule of the lens. *J Pediatr* 1977;**91**:455–8.

[10] Ryan SW, Congdon PJ, James JR, Truscott J, Horsman A. Mineral accretion in the human fetus. *Arch Dis Child* 1988;**63**:799–808.

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Association of mineral composition of neonatal intravenous feeding solutions and metabolic bone disease of prematurity

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SUMMARY To assess the effects of increasing the mineral content of parenteral nutrition solutions on the biochemical and radiological indicators of metabolic bone disease of prematurity 27 neonates who required parenteral nutrition were sequentially allocated to receive either a standard solution (group 1) or one with an increased mineral content (group 2). The 13 patients in group 1 received 0·68 mmol/kg/day of calcium and 0·61 mmol/kg/day of phosphorus, and the 14 in group 2 received 1·25 and 1·20 mmol/kg/day, respectively. The two groups did not differ significantly in the severity of their illness measured by birth weight, gestational age, duration of parenteral nutrition or ventilation, or the amount of supplementary oxygen required. In patients in group 2 the median plasma phosphate concentration was higher, the plasma alkaline phosphatase activity was lower, and there was less radiological evidence of rickets. There were no complications caused by excess calcium and phosphorus, and the rate of growth was similar in both groups. We conclude that an increased mineral content in parenteral nutrition solutions reduces the severity of metabolic bone disease in sick infants who require this form of nutrition.

Metabolic bone disease is common in premature infants whether they are fed enterally[1] or parenterally.[2] Deficiency in the mineral substrate is thought to be an important aetiological factor,[3] though increasing the intake of calcium and phosphorus in enterally fed infants failed to reduce the incidence of rickets.[4] In utero accretion of calcium occurs at about 2·75–3·25 mmol/kg/day, and phosphorus at about 2·1–2·6 mmol/kg/day[5]; conventional intravenous feeding solutions cannot deliver these minerals at these rates without making them insoluble.[6] Alternating solutions with either a high calcium or phosphorus concentration has been tried to overcome this problem,[7] but this increases the difficulty of feeding, and a fluctuating intake is not physiological.

The amount of calcium and phosphorus that can be held in solution without precipitation is decreased if the amino acid concentration is decreased.[8] Our standard parental nutrition solution normally delivers calcium and phosphorus at a rate of 0·6–1·0 and 0·7 mmol/kg/day, respectively. If higher rates are attempted, precipitation occurs if the intake of amino acid is reduced. In vitro studies have, however, shown that the intake of calcium and phosphorus can be increased to as much as 2·0 and 1·7 mmol/kg/day, respectively, without precipitation if the intake of amino acid (using Vamin-9-glucose, KabiVitrum) is maintained at 50 ml/kg/day (P MacMahon, *et al*, unpublished observations). We used this to formulate a second parenteral nutrition solution with an increased calcium and phosphorus content without altering the amino acid content. The modified solution with extra calcium and phosphorus was then compared with our previous standard in a controlled trial designed to assess the effects of the increased mineral content of the parenteral nutrition solution on the biochemical and radiological indicators of metabolic bone disease of prematurity.

Patients and methods

Between July 1985 and October 1986 every infant admitted to the neonatal intensive care unit at this hospital who required parenteral nutrition was sequentially allocated to one of two groups that received either our standard solution (group 1) or one with an increased mineral content (group 2). The prescription and formulation was aided by a modification of a previously published computer protocol from this unit.[9] The two formulas differed only in their calcium and phosphorus content. Daily intakes of calcium and phosphorus were altered according to the plasma concentration, provided that the total fluid and amino acid intake were such that precipitation would not occur (table 1). The intake of phosphorus in group 1 was not influenced by the plasma concentration, but was limited to 0·3 mmol/kg/day if the intake of calcium was more than 1 mmol/kg/day, or if the intake of fluid was less than 120 ml/kg/day, or if the intake of Vamin was less than 30 ml/kg/day.[7 8]

Criteria for inclusion in the study were that at least 75% of the volume of fluid was given intravenously, and that parenteral nutrition was required for at least 14 days. The prescription of parenteral feeding was by standard clinical criteria. When enteral feeding was possible the mother's own expressed breast milk was used in preference; if not, a proprietary preterm formula was used (Prematalac, Cow and Gate or Osterprem, Farley).

Calcium in the parenteral nutrition solution was supplied as 10% calcium gluconate (2·2 mmol/10 ml), in the trace element preparation (Pedel, KabiVitrum 1·5 mmol/10 ml) and in the amino acid preparation (Vamin-9-glucose, KabiVitrum, 0·25 mmol/100 ml). Phosphorus was supplied as 8·7% potassium phosphate (5 mmol/10 ml) and in the trace element preparation (Pedel, KabiVitrum, 0·75 mmol/10 ml). Particular attention was paid to the sequence of additions during the formulation to avoid precipitation. The phosphorus salt was the first additive to the amino acid/dextrose mixture, and any calcium salts were added last.[8] The solutions were refrigerated until required. The amount of each solution infused was recorded and from this the amount of calcium and phosphorus actually delivered was calculated.

Table 1 *Amounts of calcium and phosphorus prescribed depending on plasma concentration*

	Plasma concentration (mmol/l)	*Intake (mmol/kg/day)*	
		Group 1	*Group 2*
Calcium:	>2·8	0	0
	2·61–2·8	0	0·6
	2·41–2·6	0·6	1·0
	2·21–2·4	0·6	1·5
	2·01–2·2	1·0	2·0
	1·5–2·0	1·5	2·0
	<1·5	2·0	2·0
Phosphorus:	>2·0		0·3*
	1·51–2·0	0·7	0·7
	≤1·5		1·7†

*Minimum intake if estimations of trace elements requested.
†Limited to 1·2 if intake of Vamin=40 ml/kg/day and to 0·7 if intake of Vamin <40 ml/kg/day.

The mineral content of expressed breast milk was assumed to be 7·0 mmol/l of calcium and 4·8 mmol/l of phosphorus with an average absorption rate of 34 and 86% respectively.[10] The mineral content of any proprietary milk used was calculated from the manufacturer's data sheet and the percentage absorption of calcium and phosphorus was assumed to be 42 and 82·5, respectively.[10] From these data the amount of calcium and phosphorus absorbed from any enteral feeds was calculated.

The plasma concentration of calcium was measured 48 hourly, or more frequently if clinically indicated. The plasma concentration of phosphate and the alkaline phosphatase activity were measured at least weekly. These assays were done on an American Parallel Monitor using approved methods. Standardised radiographs of the wrists were taken every two weeks, and subsequently reviewed by a single observer who was unaware of which group the patient was in (PT). They were each given a radiological score using the method of Koo *et al* in which the classical radiographic changes of serious rickets equalled grade 2.[11]

Parenteral nutrition was normally infused through a silicone catheter (Vygon) inserted percutaneously, the tip of which was placed in a large central vein (axillary vein, superior or inferior vena cava) to make it easier to give high dextrose concentrations (greater than 10%). When this was not technically possible (and also during episodes of suspected septicaemia) a peripheral vein was used. In infants who were particularly unstable and in whom an umbilical arterial catheter was already in situ, this was used.

Measurements of weight, head circumference, and length were recorded weekly using an electronic scale, graduated tape measure, and measuring board respectively.

Statistical analysis of the data was by the Statistical Package for the Social Sciences (SPSS-X) computer program using Student's *t* test to assess significance of differences for parametric data, and the Mann-Whitney U test for non-parametric data (including the radiological scores). The study was approved by the hospital ethics committee.

Results

Twenty seven infants (13 in group 1 and 14 in group 2) were enrolled in the study. At birth there were no significant differences between the groups for gestational age, birth weight, or sex. Subsequently there were no differences between the groups in their severity of illness as measured by the durations of parenteral nutrition, ventilator support, or the amount of supplementary oxygen required. Parenteral nutrition was usually fully established after seven days, and only the results obtained after this time were analysed. There were no differences between the groups in the daily intravenous intake of protein, fat, or carbohydrate, and the energy and mineral contents of the small quantities of enteral feeds (table 2).

The intakes of copper (0·3 μmol/kg/day supplied in Pedel, KabiVitrum), zinc (0·6–μmol/kg/day also supplied in Pedel, KabiVitrum), and vitamin C (3·4 mg/kg/day of sodium ascorbate supplied in Solivito, KabiVitrum) were identical in both groups.

The modified parenteral nutrition regimen achieved its primary objective of delivering an increased amount of mineral substrate to the 14 infants in group 2. Group 1 received 0·68 mmol/kg/day of calcium and 0·61 mmol/kg/day of phosphorus whereas group 2 received 1·25 and 1·20, respectively (table 2).

The plasma calcium concentration remained normal throughout the study with the mean weekly value remaining within the range 2·3–2·5 mmol/l in both groups. In group 1 the plasma phosphate concentration was lower, the plasma alkaline phosphatase activity was higher and the radiological scores for rickets were higher while the infants remained on parenteral nutrition (figure). The only three infants to develop classical radiographic changes of rickets (grade 2) were all in group 1.

There was no difference in the rate of growth between the groups measured by the weekly mean percentage increase in weight, length, or head circumference. Comparing group 1 with group 2 after six weeks on parenteral nutrition the weight gain was 53·3% compared with 45·2% (p=0·55), the increase in length was 8·7% compared with 13·4%

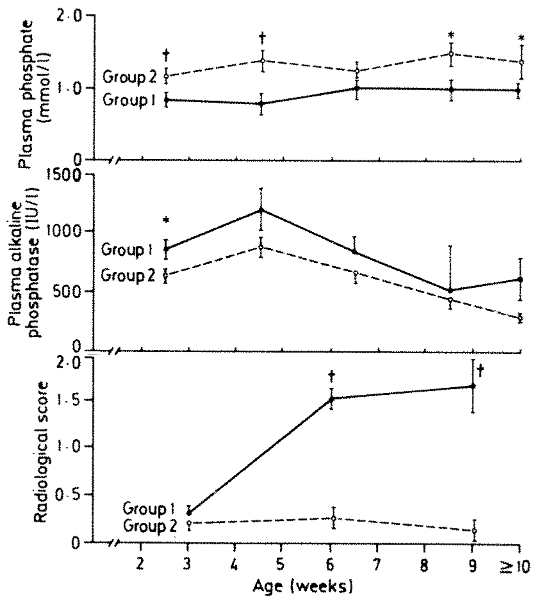

Figure *Plasma phosphate concentration, alkaline phosphatase activity, and radiological score with increasing age. Vertical bars are mean (SEM); *=p value ≤0·1 and †=p value ≤0·05 (Mann-Whitney U test).*

Table 2 *Clinical characteristics and mineral intakes of infants studied expressed as median (range)*

	Group 1	*Group 2*	*p Value*
Birth weight (g)	960 (580–1760)	830 (590–1495)	NS
Gestational age (weeks)	28 (25–33)	26 (24–41)	NS
Male:female ratio	7:6	8:6	NS
Duration of:			
Parenteral nutrition (days)	48 (14–199)	56 (14–77)	NS
Intermittent positive pressure ventilation (days)	48 (0–102)	40 (2–83)	NS
Supplementary oxygen (days)	18 (0–51)	16 (0–75)	NS
Parenteral intake:			
Energy (MJ/kg/day)	0·360 (0·176–0·594)	0·360 (0·142–0·523)	NS
Protein (g/kg/day)	3·43 (1·05–4·2)	3·49 (0·2–4·2)	NS
Fat (g/kg/day)	2·2 (0·4)	2·1 (0–4·1)	NS
Dextrose (g/kg/day)	13·5 (6·8–23)	13·2 (3·1–20·1)	NS
Enteral intake:			
Energy (MJ/kg/day)	0·004 (0–0·146)	0·008 (0–0·155)	NS
Calcium (mmol/kg/day)	0 (0–0·2)	0·02 (0–0·4)	NS
Phosphorus (mmol/kg/day)	0 (0–0·4)	0·02 (0–0·81)	NS
Total mineral intake:			
Calcium (mmol/kg/day)	0·68 (0·32–1·05)	1·25 (0·29–1·84)	<0·001
Phosphorus (mmol/kg/day)	0·61 (0·16–0·98)	1·20 (0·39–1·74)	<0·001

(p=0·09), and the increase in head circumference was 10·3% compared with 15% (p=0·16).

Discussion

We have shown that it is possible to increase significantly the calcium and phosphorus content of intravenous feeding solutions prescribed for preterm neonates. Rickets of prematurity is a recognised complication of parenteral nutrition in these babies[2]; and mineral deficiency is thought to be an important factor in the aetiology of metabolic bone disease in infants[3 5]; it is well recognised in association with prolonged parenteral nutrition.[2 12] If calcium and phosphorus are added to conventional parenteral nutrition solutions in the concentrations necessary to maintain normal transplacental transfer and accretion,[5] precipitation of calcium and phosphate crystals usually occurs.[6] Because of this, most regimens limit the delivery of these minerals to less than 1 mmol/kg/day of each.

The factors that increase the likelihood of precipitation of calcium and phosphorus in parenteral nutrition solutions include high molar concentrations, high pH of the solution, low concentrations of amino acid, high temperature, prolonged standing times, adding the calcium salts either first or before the final dilution, and the use of the chloride salt as a source of calcium.[8] By prescribing increased amounts of calcium and phosphorus only when the fluid and amino acid intakes were appropriate, precipitation was avoided. The increased plasma phosphate concentrations in group 2 suggest that the increased mineral content is retained with the higher formulation.[10]

Plasma alkaline phosphatase activity is a clinically practical screening test for significant demineralisation[13]; biochemical rickets frequently precedes the typical radiological changes. Occasionally the alkaline phosphatase activity is in the normal range even in radiologically apparent rickets.[14] Photon absorptiometry provides an accurate quantification of demineralisation,[15] but this method was not available to us. The radiological scoring system developed by Koo *et al* does, however, also permit an objective grading of osteopenia (to rickets) as well as comparison between infants, and is particularly useful in the diagnosis and subsequent monitoring of progress to healing.[11]

The lower plasma alkaline phosphatase activity and better radiological outcome in group 2 suggests that overall bony mineralisation was improved compared with the group that received less mineral supplement. This outcome cannot be explained by differences in intake of other minerals, such as copper and zinc, as there was no difference in these between the groups.

Our results indicate that it is possible to increase the calcium and phosphorus content of intravenous feeding solutions significantly to match more accurately the intrauterine accretion rates, and that this reduces the incidence and severity of metabolic bone disease in preterm infants who require this form of nutrition. Metabolic bone disease can exacerbate bronchopulmonary dysplasia[16] as well as cause respiratory problems in preterm infants with no other identifiable lung disease.[17] Muscle weakness secondary to phosphorus depletion may also cause additional complications.[18] Improved skeletal mineralisation should therefore help to alleviate the chronic respiratory problems that are common in these infants.

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References

[1] McIntosh N, Livesey A, Brooke OG. Plasma 25-hydroxy vitamin D and rickets in infants of extremely low birthweight. *Arch Dis Child* 1982;**57:**848–50.

[2] The TS, Kollee LAA, Boon JM, Monrents LAA. Rickets in a preterm infant during intravenous alimentation. *Acta Paediatr Scand* 1983;**72:**769–71.

[3] Brooke OG, Lucas A. Metabolic bone disease in preterm infants. *Arch Dis Child* 1985;**60:**682–5.

[4] McIntosh N, De Curtis M, Williams J. Failure of mineral supplementation to reduce incidence of rickets in very-low-birthweight infants. *Lancet* 1986;**ii:**981–2.

[5] Greer FR, Tsang RC. Calcium, phosphorus, magnesium and vitamin D requirements for the preterm infant. In: Tsang RC, ed. *Vitamin and mineral requirements in the preterm infant*. New York: Marcel Dekker, 1985:99–136.

[6] Poole RL, Rupp CA, Kerner JA. Calcium and phosphorus in neonatal parenteral nutrition solutions. *Journal of Parenteral and Enteral Nutrition* 1983;**7:**358–60.

[7] Knight PJ, Buchanan S, Clatworthy HW Jr. Calcium and phosphate requirements of preterm infants who require prolonged hyperalimentation. *JAMA* 1980;**243:**1244–6.

[8] Niemiec PW Jr, Vanderveen TW. Compatability considerations in parenteral nutrient solutions. *Am J Hosp Pharm* 1984;**41:** 893–911.

[9] MacMahon P. Prescribing and formulating neonatal intravenous feeding solutions by microcomputer. *Arch Dis Child* 1984;**59:** 548–52.

[10] Lyon AJ, McIntosh N. Calcium and phosphorus balance in extremely low birthweight infants in the first six weeks of life. *Arch Dis Child* 1984;**59:**1145–50.

[11] Koo WWK, Gupta JM, Nayanar VV, Wilkinson M, Posen S. Skeletal changes in preterm infants. *Arch Dis Child* 1982;**57:** 447–52.

[12] Ricour C, Millot M, Balsam S. Phosphorus depletion in children in long term parenteral nutrition. *Acta Paediatr Scand* 1975;**64:** 385.

[13] Kovar IZ, Mayne P, Barltrop D. Plasma alkaline phosphatase activity: a screening test for rickets in preterm neonates. *Lancet* 1982;**i:**308–10.

[14] McIntosh N. Rickets of prematurity. *Bone* 1984;**1:**26–7.

[15] Cameron JR, Mazess RB, Sorenson JA. Precision and accuracy

of bone mineral determination by direct photon absorptiometry. *Invest Radiol* 1968;**3**:141–5.

[16] Geggel RL, Pereira GR, Spackman TJ. Fractured ribs: unusual presentation of rickets in premature infants. *J Pediatr* 1978;**93**: 680–2.

[17] Glasgow JFT, Thomas PS. Rachitic respiratory distress in small premature infants. *Arch Dis Child* 1977;**52**:268–74.

[18] Schott GD, Wills MR. Muscle weakness in osteomalacia. *Lancet* 1976;**i**:626.

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Perinatal lessons from the past

> 'Is there anything whereof it may be said,
> See, this is new?
> It hath been already of old time,
> Which was before us'
>
> *Ecclesiasticus, 1, 10*

In 1963 the late Professor A V Neale of Bristol read to us from William Smellie's famous *Midwifery* (1752) during a ward round. For me this was the start of a voyage of excitement and delight into medical history. It is in the hope that others will be stimulated to share this pleasure that the Editors agreed to my preparing a series of articles of which the first concerns James Blundell and neonatal resuscitation (p 494). My purpose will not be confined to illustrating the accuracy of the quotation from *Ecclesiasticus* shown above. I hope also from time to time to reveal the truth of a remark made by Sir Robert Hutchison during his Harveian Oration in the Royal College of Physicians in 1931:

'Look round this room in which we are met. It is a noble library indeed, but is it not also a mausoleum? And how many facts which men are at present hunting for, and theories which are even now being put forward as new, lie already buried in these shelves'.

The importance of a knowledge of medical history is also clearly drawn in the following lines written in 1925 by that wise paediatrician, historian, and philosopher, Dr John Ruhräh of Baltimore:

'The unread physician is like a man in the theater without a program or a libretto or often, perhaps, without even a knowledge of the language of the stage. He is like a deaf man at an opera. Someone who has been watching the play before he came in may tell him something about it; who the principal characters are; who it is that sings so sweetly, so convincingly. He may gain a slender knowledge of the plot from what the actors say or do, and that is all. The jester enters, makes a loud noise with an empty bladder on the end of a stick, tweaks the nose of the king or his chancellor, cracks his time-honored jest and vanishes amid the laughter and applause of the delighted, if uninitiated, audience. Character after character appears and disappears, most of them the same old familiar faces, though sometimes with new names and new costumes. The auditor gasps and wonders. A timid voice speaks a great truth, but no one pays attention and presently the stage manager pulls the speaker from the stage while the clown and the trained dogs hold the attention of the audience.

A century or two later, the same truth is told again, but now the spotlight is on the handsome actor, with a wonderful voice, mouthing his nothings most delightfully. Again the audience pays no attention, but after many repetitions, the stage is set for Truth and, amid great applause, some one or a group parade Her with great pomp, as if for the first time. The audience gasps again and asks why She was not brought out before: so wonderful She is! Had he but known, had he paid attention—he had heard it long ago, but Now it is the wonderful, new thing. But perhaps it was not on the stage while he was in the audience. Well, it was set down in the account of the play, but he was too indolent, too busy, or else too lazy to read it.'

P M DUNN

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Perinatal lessons from the past

Dr James Blundell (1790–1878) and neonatal resuscitation

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James Blundell, MD, was born in 1790. He graduated in Edinburgh in 1813 and from 1814 until 1836 he taught midwifery at Guy's Hospital where he became Professor of Obstetricy. Besides being the most distinguished British accoucheur of his day he was also a leading physiologist, making important contributions to the field of abdominal surgery, to the understanding of the origin of puerperal sepsis, and in the use of blood transfusion for postpartum haemorrhage. In 1838 he was made a Fellow of the Royal College of Physicians, a rare honour at that time for an obstetrician. His misfortune was that in many respects he was in advance of the technical possibilities of his day. However, having taken early retirement he lived to a grand old age and saw the introduction into medicine of many advances for which he had paved the way. Blundell's obstetric lectures were published in 1834.[1] This short extract on the endotracheal intubation and artificial respiration of severely asphyxiated infants may surprise those who believe that this technique was only introduced 20–30 years ago.

Figure *Dr James Blundell of Guy's Hospital, 1790–1878.*

'You will sometimes find that children are still-born, as it is called, that is, although they are not dead, they do not cry, or manifest other indications of life . . . In performing artificial respiration on new-born children, I have frequently observed, that while the respiration was continued, the chord pulsated, ceasing to beat in a few seconds, when the operation was suspended, and this repeatedly. These facts admitted, there can, I presume, be no doubt, that when the foetus is still-born, the artificial respiration should be diligently tried . . . The only mode of performing this operation effectually is by means of a small instrument, the tracheal pipe, which I think every accoucheur should carry along with him to a labour. The tracheal pipe is a little tube of silver, designed to pass into the trachea, its end closed like a catheter, with a long, broad fissure on either side to give free vent to air and mucus. The closed extremity and lateral openings I prefer, as there is less risk of injuring the delicate membrane of the trachea, if a terminal aperture do not exist. In introducing this instrument there is some difficulty at first, if you do not manoeuvre rightly; yet every moment is of the greatest importance, for while you are blundering the child is dying. Now not to detain you needlessly, I may be allowed to observe, that my own method of operating is the following:— I pass the fore-finger of my left hand down upon the root of the tongue and into the rima glottidis, and then using the tube with the right hand, I slide it along the surface of the finger, used as a director, till reaching the rima I insert the tube at the moment when the finger is with-drawn from it, afterwards feeling on the front of the neck whether the instrument is lying in the trachea or the oesophagus. This done, you may take the child into your hands, and from your own lungs you may inflate the lungs of the foetus, emptying them afterwards by means of double pressure of the hand, on the thorax I mean, and the abdomen, the latter pressure being necessary in order to urge upwards the diaphragm. Operating in this manner, you may execute the artificial respiration with the best success. Five-and-twenty, or thirty respirations there ought to be in a minute, the new-born child breathing faster than an adult. You may ask me, perhaps, whether it would not be better to use bellows? Make the experiment, and you will not repeat the question. When you have performed the artificial respiration for a few minutes, you make your observations on the child. Feel the chord, and you will sometimes have the satisfaction to find it pulsate. The best point for examination is at the very root of the funis, close to the abdomen. You sometimes feel the pulsation there; when at the distance of an inch from the abdomen it cannot be perceived, the arteries being so contracted that they do not admit the entrance of the blood. Examine the thorax, feel the heart, and you may sometimes, through the ribs, obscurely perceive its beating; observe the face, perhaps you find the cheeks reddening—the countenance forming—the lips quivering. When these marks of returning life are observed, pause for a little, and frequently the child will be observed to make a spontaneous effort of respiration; a deep sigh is the first breath it draws; in twenty or thirty seconds it breathes again. Now if on suspending the artificial respiration the heart continue to beat—the chord to pulsate—and the respirations to increase in frequency—further aid from the tube will not be required; but should the pulsation cease in the chord, and the sighs be heard on longer, then your operations must be resumed; and thus repeatedly, as the case requires; at one time you try the natural powers of the child, at another you support the respiration by art . . . Never hastily despair of the means of resuscitation. Many a foetus is laid aside as dead which, by a diligent use of resuscitants, might have been saved. A woman, run over by a stage, was carried into St Thomas's Hospital, and died in a few minutes after admission. This woman was in the end of pregnancy. By my friend, Mr Green, I was requested to assist in the Caesarian operation. In thirteen minutes from the last respiration of the mother, the child was taken out. In fifteen minutes from the last repiration of the mother, I began the artificial respiration. During fifteen minutes longer I continued it, ultimately resuscitating the child completely, and had due care been taken it would probably have been living still.'

Reference

[1] Blundell J. *Principles and practice of obstetricy*. Edited by T Castle. London: E Cox, 1834:246–50.

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Personal practice

Follow up studies during the first five years of life: a pervasive assessment of neurological function

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> . . . indeed, it is not easy to be accurate in an account of anything, however simple. Zoologists often disagree in their descriptions of the curve of a shell, or the plumage of a bird, though they may lay their specimen on the table, and examine it at their leisure; how much greater becomes the likelihood of an error in the description of things which must be in many parts observed from a distance, or under unfavourable circumstances of light and shade; and of which many of the distinctive features have been worn away by time. I believe few people have an idea of the cost of truth in these things; of the expenditure of time necessary to make sure of the simplest facts, and of the strange way in which separate observations will sometimes falsify each other, incapable of reconcilement, owing to some imperceptible inadvertency.[1]

These words were written by Ruskin in 1851, but they express precisely the problem that research workers face today when defining measures of outcome for use in follow up studies. Ruskin's comments have particular relevance to the collection and recording of data concerning the constellation of disorders generally referred to as 'cerebral palsy'.[1]

In the past, cerebral palsy was the commonest neurodevelopmental impairment reported in follow up studies of low birthweight or very preterm infants.[2 3] In particular, spastic diplegia was generally regarded as a typical condition among infants whose neonatal courses were complicated by apnoea and bradycardia. These impairments were believed to be so typical in infants who survived before the introduction of modern neonatal intensive care that it was proposed that their prevalence could be used as a measure of the quality of survival for audit, and comparative and epidemiological studies.[4] As interest widened and large epidemiological studies were planned, the need for a universally agreed, simple definition of cerebral palsy was recognised; agreement proved difficult. Various suggestions were made ranging from a simple description based on Little's original proposal,[5 6] to a complex standardised physiopathological classification of syndromes of cerebral palsy that was put forward by paediatric neurologists.[7] Interobserver agreement was studied and found to be poor according to either type of definition.[8] Longitudinal studies also indicated that the diagnosis of cerebral palsy was not consistent over time in individual children, at least not until they were older, and aged 5 years or more.[9] As a result of these observations warnings were expressed that perhaps cerebral palsy was unsuitable to use as an outcome measure, particularly among young children.[9 10]

While perceptions of the nature of cerebral palsy and its use as a measure of outcome were changing, so also were the circumstances in which the condition occurred. For example, as methods of neonatal care were progressively refined, so the pattern of insults to the newborn brain changed in character and extent. Many potentially damaging events were avoided, or at least ameliorated, although homoeostasis was not guaranteed. Indeed, infants began to survive after insults that had previously been thought to be fatal. As a consequence there was a change, both in the type of infants surviving and the brain lesions that they suffered.[11] For example, in a recent study even using the most liberal definition, cerebral palsy seemed to affect a minority of the neurologically impaired and disabled children.[12] Thus to describe the outcome of a group of infants solely according to the prevalence of cerebral palsy is both inadequate and misleading.

Proposals

We believe the problem could be overcome by describing the neurological condition of each infant objectively according to clearly defined standards. This approach would eliminate the need to interpret findings to fit them into specific diagnostic categories, and it is likely that it would also overcome the problem of the apparent variation of diagnosis with age in individual children. There is already evidence indicating that it is the manifestations of brain damage that vary according to age, and not the underlying lesions.[13–17] For example, infants with neurological impairments in the first year of life have been reported to have significantly poorer scores in tests of cognitive function than their unaffected peers,[17 18] and they are believed by some to be particularly liable to present with learning difficulties as they grow older.[13 15 16 18 19] Thus these children represent an aspect of perinatal brain damage that is important as an index of outcome, but which may not be recognised as such because the early neurological signs seem transient and the learning difficulties are not identified until the children are much older and the early signs have been forgotten. In fact the transience of the early neurological signs is probably apparent rather than real. It is more likely that the signs are present but are often not detected because of the power of the toddler to defy neurological assessment between the ages of about 2 and 4 years.

Methods

The scheme we propose is designed to record the consequences of brain damage in a purely objective manner. Objective neurological information is obtained from a structured neurological examination using standardised procedures to elicit the signs and to judge the findings. Throughout the first year of life the scheme and standards for neurological examination already described by one of us may be used.[20] The scheme includes detailed instructions for eliciting the signs. The same principle and methods may be applied from the second year of life using age-appropriate standards to judge the findings. Experience has shown that by the end of the first year of life all major impairments can be detected, and any persisting minor impairments must be regarded as significant. Thus categorisation of neurodevelopmental condition at this age may have both meaning and predictive value.[17] We therefore give here standards starting from the age of 9 months (table 1) although standards for use throughout the first year are available elsewhere.[20] A record form for the neurological data that are obtained at each examination is provided (table 2). We recommend that each child should have an individual record form. Findings should be recorded on this form either according to the presence or absence of a sign or as absolute measurements of angles in degrees. The findings may then be judged according to the standards appropriate for age given in table 1 and added to the results of assessments of hearing, vision, and behaviour, measurements of head growth, and of psychometric testing, to give a comprehensive assessment of a child at a particular age. The choice of methods for tests of hearing and vision, and of psychometric assessments, depends on the age of the child and the availability of the tests.

Findings judged to be normal because—for example, the size of an angle or a test score falls within the range appropriate for age—may be coded 0. Findings judged to be mildly deviant on the basis of angle measurements or test scores may be coded 1, and severely deviant findings may be coded 2. This coded information may be recorded on the chart given in table 3 to provide a summary of all the findings in an individual child at a particular age. When making this summary a single mildly deviant item (coded 1) may be ignored. Two or more mildly deviant items (giving a total of 2 or more) indicate abnormality, and severely deviant items (coded 2) always indicate abnormality, even if there is only one item in this category. Finally, on the basis of this coded information, neurodevelopmental condition can be described as 'unimpaired', 'impaired, but without disability' (mildly deviant), or 'impaired with disability' (severely deviant).

Because the type of records that we propose may form the basis of a minimum set of data for an individual child that may be used to monitor progress or to provide information for research, provision has been made on the summary form (table 3) for recording some additional facts, under the heading 'other information'. These include conditions which, although not necessarily developmental in origin, may influence a child's progress: they can also be used to provide a wider definition of abnormality if required. Examples include refractive errors, chronic lung or gastrointestinal disease, and growth.

THEORETICAL BASIS OF THE SCHEME FOR NEUROLOGICAL EXAMINATION

In an earlier report Amiel-Tison described a waxing and waning pattern of neuromotor development from 28 weeks' gestation to the end of the first year of life.[21] This description was based on the clinical evaluation both of posture and passive tone at rest, and of motor activity and postural reactions; it conformed with proposals originally made by both Thomas and Saint-Anne Dargassies,[22] and by Peiper.[23] For example, from 28 to 40 weeks' gestation the acquisition of muscle tone and motor

Table 1 *Standards for coding neurological data*

	9–17 months	*18–60 months*
1 (a) **Neuromotor: hypertonia**		
Arms:		
Scarf		
Anterior axillary line to midline	1	1
Less than anterior axillary line	2	2
Candlestick posture*: present/fixed	2	2
Hand flapping:		
tightening	1	1
nearly absent	2	2
Legs:		
Adductor angle 110°–40°	1	1
<40°	2	2
Heel-ear angle 110°–100°	1	—
100°–90°	—	1
<100°	2	—
<90°	—	2
Popliteal angle 110°–100°	1	1
<100°	2	2
Ankle angle 80°–90°	1	1
>90°	2	2
Ankle stretch response—		
phasic	1	1
tonic	2	2
clonus	2	2
Axis:		
Imbalance of tone		
Excessive extension *v* flexion	1	1
Permanent opisthotonus	2	2
Falls back from sitting—present	2	2
'Scissoring', held up—present	2	2
1 (a) **Neuromotor: hypotonia**		
Arms:		
Scarf:		
Minimal resistance	—	1
Flaccid (no resistance)	2	2
Hand-flapping—flaccid (no resistance)	2	2
Legs:		
Adductor angle:	Impossible to define	Impossible to define
Heel-ear angle:	Impossible to define	—
>150°	—	1
Popliteal angle:	Impossible to define	—
>160°	—	1
Ankle angle:	Impossible to define	Impossible to define
Axis:		
Global hypotonia (excessive flexion and extension):		
'Rag doll'†	2	2
Falls forward, sitting—present	2	2
Supporting/standing response—feeble/nil	2	2
1 (a) **Reflexes**		
Limbs:		
Tendon—very brisk	1	1
clonus	2	2
Primitive—asymmetrical tonic neck reflex present	2	2
1 (a) **Reflexes** (cont.)		
Postural—lateral propping/parachute		
Nil at 10 months	1	—
Nil at 12 months	2	—
Hesitant	—	1
Absent	—	2
Hands—fisting/cortical thumbs, tendency	1	1
fixed	2	2
1 (a) **Asymmetry**		
Asymmetrical findings (difference of ≥1 grades or ≥10° passive tone or both)	1	1
1 (a) **Cranial nerves**		
Abnormal	1	1
1 (b) **Neuromotor**—consequences at examination—record abnormality as 'yes'		
Head control: imperfect or absent		
Sitting: unstable—falls forward or back — or absent		
Standing: unsteady or absent		
Walking: few steps or absent after 18 months		
Gait: 'inelegant' or abnormal		
Use of right upper limb impaired		
Use of left upper limb impaired		
Use of right lower limb impaired		
Use of left lower limb impaired		
2 **Neurosensory**		
Hearing:		
Sensory loss, high tone, present	1	1
Sensory loss speech frequencies, present	2	2
Eyes/vision:		
'Fix-track' behaviour:		
Poor, brief, one plane only, or absent	2	2
Nystagmus or roving eye movements: present	2	2
3 **Neurobehavioural**		
Seizures: with or without treatment, present	2	2
Hyperexcitability: one or all present	1	1
Lethargy: one or all present	1	1
Involuntary movements: present	2	2
Sucking or swallowing: absent	2	2
Drooling: present	1	1
4 **Head growth**		
Head circumference:		
Two standard deviations above or below the mean	2	2
Growth velocity: <10 or >90 centile	2	2
Ventriculoperitoneal shunt: present	1	1
5 **Psychometric tests—define abnormality according to test instructions—for example:**		
McCarthy scale of children's abilities:		
General Cognitive Index—70–79: Impaired		
General Cognitive Index—<70: Impaired with disability		

*Upper arm in line with shoulder with maximum supination and flexion to 90° at elbow; †very floppy with unlimited extensibility.

Table 2 *Individual record form for neurological data*

Section				*1*	*2*	*3*	*4*	*5*
1 **Passive tone**	Upper limb:	Scarf sign	Value					
		Candlestick*	No/yes					
		Hand flapping	OK/not					
	Lower limb:	Adductor angle	Value					
		Heel-ear angle	Value					
		Popliteal angle	Value					
		Ankle angle	Value					
		Ankle stretch response	Type					
	Axis:	Ventral flexion	OK/not					
		Dorsal extension	No/yes					
		Opisthotonic posture	No/yes					
1 **Active tone**	Head control:		Yes/no					
	Sitting:		Yes/no					
		Falls backwards	No/yes					
		Falls forward	No/yes					
	Standing:		Yes/no					
		'Scissoring'	No/yes					
	Walking:		Yes/no					
	Gait:		OK/not					
	Use of lower limbs:		Yes/no					
1 **Reflexes**	Primitive:	Moro response	No/yes					
		Asymmetrical tonic neck reflex	No/yes					
	Tendon:	Biceps	OK/not					
		Knee	OK/not					
		Ankle	OK/not					
	Clonus:		No/yes					
	Protective:	Propping	Yes/no					
		Parachute	Yes/no					
	Hands (right/left):	Fisting	No/yes					
		Cortical thumbs	No/yes					
	Use of upper limbs and hands:		OK/not					
1 **Asymmetry**	Arms/legs/axis:		No/yes					
1 **Cranial nerves**			OK/not					
2 **Ears**	Pure tone audiogram (SOM/HTHL/SNHL)†:		OK/not					
2 **Eyes**	'Fix'/'track' behaviour:		OK/not					
	Nystagmus/roving movements:		No/yes					
	Visual field:		OK/not					
	Visual acuity (right/left):		Value					
	Squint:		No/yes					
	Sunset:		No/yes					
3 **Seizures** (treated or not)			No/yes					
3 **Hyperexcitability**								
	Tremor/clonic movements:		No/yes					
	Sleep disorders:		No/yes					
	Excessive crying:		No/yes					
3 **Lethargy**	Reduced activity:		No/yes					
	Drowsy:		No/yes					
	Infrequent crying:		No/yes					
3 **Involuntary movements**								
	Upper limbs:		No/yes					
	Lower limbs:		No/yes					
3 **Sucking/swallowing**			OK/not					
3 **Drooling**			No/yes					
4 **Head growth**	Head circumference (cm):		Value					
	Velocity (centile):		Value					
4 **Ventriculoperitoneal shunt**			No/yes					

*Upper arm in line with shoulder with maximum supination and flexion to 90° at elbow; †secretory otitis media, high tone hearing loss, sensory neural hearing loss. Full details of procedures to elicit neurological signs have been previously published.[20]

Table 3 *Record form to summarise neurodevelopmental state at any age within the first five years of life*

Name .... Date of birth .... Date of test .... Gestational age .... Corrected age ....

1 **Neuromotor** (tone and reflexes)

(a) Findings:

Axis:	Global hypotonia	☐
	Imbalance (extensor>flexor)	☐

		Right		Left	
		Arm	Leg	Arm	Leg
Limbs:	Hypertonia				
	Hypotonia				
	Asymmetry			/	/
	Exaggerated tendon reflex				
	Abnormal hands		/		/
	Abnormal postural reflex				

(b) Consequences at examination:

	Yes	No
Head control		
Sitting		
Standing		
Walking		
Normal gait		
Use of right upper limb/hand		
Use of left upper limb/hand		
Use of right lower limb		
Use of left lower limb		

2 **Neurosensory**

Hearing loss	☐
Nystagmus	☐
Cortical blindness	☐

3 **Neurobehavioural**

Seizures (treated or not)	☐
Irritability	☐
Depression	☐
No sucking	☐
Drooling	☐
Abnormal cranial nerves	☐

4 **Head growth**
Occipitofrontal circumference:

<two SD	☐
>two SD	☐
Ventriculo-peritoneal shunt	☐

Velocity

Cm/year	☐
Centile	☐

5 **Psychometric tests**

Subscales	Value	Standard score
1		
2		
3		
4		
5		
Overall		
Achievement score		

Conclusions

1 Neuromotor	
(a) impairment	☐
(b) function affected	☐
2 Neurosensory	☐
3 Neurobehavioural	☐
4 Head growth	☐
5 Psychometric tests	☐

Normal ☐ **Impaired** ☐ **Impaired with disability** ☐

Other information

1 Severe refractive error or retinopathy, or both	☐
2 Squint	☐
3 Conductive hearing loss	☐
4 Chronic lung disease	☐
5 *Chronic gastrointestinal disease	☐
6 Growth problem	☐
7 Congenital malformation	☐
8 Socioeconomic deprivation	☐
9 Other	☐

Normal: a total of 1 or less in sections 1–4 and scores in section 5 within the normal range for the test used. **Impaired without disability:** a total of 2 or more in section 1 (a) and no functional consequences recorded in 1 (b); and/or a total of 1 in sections 2, 3, and 4, and/or scores in section 5 within the 'doubtful' range. **Impaired with disability:** a total of 2 or more in section 1 (a) with functional consequences recorded in 1 (b), and/or 2 or more in sections 2, 3, and 4, and/or scores in the abnormal range for the test used in section 5, or a combination of these. **Other information** is not scored or used in the conclusions.
*=usually after necrotising enterocolitis.

function spreads from the lower extremities in the direction of the head. After full term the process is reversed so that relaxation and the development of motor control proceed downwards for the next 12 to 18 months. Thus the upper limbs and extremities begin to relax and acquire skills before the lower limbs, and in the axis head control is acquired first, followed by the ability to sit, to stand, and finally—to walk. The timing of this sequence is subject to wide individual variations in addition to familial and ethnic influences, but the order is constant in all normal individuals. Thus from a knowledge of the successive stages of maturation throughout the sequence it is possible to detect abnormal clusters of responses that may be used to identify deviant development. Using these observations a scheme for the evaluation of neurological development during the first year of life was designed.[20] In practice this scheme may be applied up to the age of 18 months. We believe that this is justified because, owing to wide individual variations, the sequence culminating in independent walking is often not completed by the end of the first year of life in infants who later prove to be normal. Independent walking, however, should be achieved by 18 months; failure to do so usually indicates abnormality.[24]

The orderly progression of neuromotor development continues after the first year though the changes are more subtle and result largely from improvements in motor organisation. The advances in motor control that result may be identified and assessed,[25] and passive tone at rest may still be measured. Thus the same type of assessment based on observation and manipulation of the child that is used to evaluate progress in the first year of life may be applied as the child grows older.

In order to derive standards from the second year of life we considered both the original data of Saint-Anne Dargassies[26] (that were obtained by examination of a group of normal infants followed up to the age of 2 years) and the data from our own follow up studies of infants born at full term[13 27] and those born very preterm (Stewart *et al*, unpublished observations).[12 17 28 29] Our own findings were in agreement with the standards for neuromotor function proposed by Saint-Anne Dargassies[26] up to the end of the second year of life. Thereafter we observed little change with age in posture and passive tone at rest, or in motor activity and postural reactions. We therefore consider that standards for these measures remain more or less constant from the end of the second year to the age of 4 or 5 years, and in practice can be applied from the age of 18 months when the early standards cease to apply.

When examining our own data we noted that the range of normal variation was as wide in the older children as had been observed during the first year of life in the earlier studies.[20] To take account of this observation the definitions of abnormality have to be fairly stringent to avoid including minor variations that may be of little consequence.

EXPERIENCE OF THE SCHEME IN USE

We have been using the scheme for neurological examination[20] and teaching it to paediatricians and physiotherapists for many years. Familiarity with examining and handling infants and young children is the only essential requirement for acquiring competence, but a basic knowledge of paediatric neurology helps with interpretation as it permits the examiner to extend the examination if there are abnormal or equivocal findings. We have found that successive generations of research fellows of variable experience have quickly become skilled with good interobserver reliability, a matter that is discussed in more detail elsewhere.[20]

The neurological examination takes about 10 minutes to carry out. Though it has been designed to be objective as far as possible, there are a few circumstances in which particular caution must be exercised when interpreting the findings.

Wide individual variation in passive tone makes the definition of mild symmetrical abnormalities difficult; as a consequence these conditions are probably underestimated. By contrast, asymmetry is usually accurately recognised though it does not necessarily indicate that one side is normal.

Struggling with defiant, uncooperative children may affect the precision of the examination. This tends to be a problem when examining 2–3 year olds who resent procedures designed to elicit passive tone, such as the 'scarf' sign, and occasionally their behaviour prevents completion of the examination. When struggling does occur, results will always be biased in the direction of hypertonicity.

Postural deformities secondary to prolonged immobilisation during intensive care may affect some measurements. For example, shortening of upper limb girdle muscles or of the extensor group of neck muscles may occur during prolonged periods of mechanical ventilation. This may mimic increased passive tone and lead to difficulties in interpretation.[21] Likewise, 'scissoring' within the first year may have similar postural origins. In this circumstance the sign does not necessarily support a diagnosis of spastic diplegia.[30] In general when such findings are isolated ones they are more likely to be postural than central.

Conclusions and implications

In clinical practice, follow up teams may use this scheme to help them offer continuous care to infants and their parents from the time the infants go home. If the findings are normal it will be possible to reassure many parents at an early stage,[29] and infants with impairments may be recognised so that treatment can be started and help provided for the parents as soon as possible. Infants with movement disorders or cerebral palsy will be included among those with neurological impairments and treated as soon as they are recognised, though they may not be labelled precisely until years later.

In research the scheme provides objective data that can be used in longitudinal studies and analysed in detail, item by item. In epidemiological studies these data permit categorisation of neurodevelopmental condition without the need to use any physiopathological nosology. Thus it should be possible to trace the influence of perinatal events right through infancy and the preschool period to the age when the diagnosis of learning difficulties and other school problems is possible.

Difficulties are inherent in the examination of the preschool child; they cannot be ignored or bypassed. From our own experience, however, we believe that an analytical approach to the assessment of neurological function is feasible, with interobserver reliability and consistency over time in an individual child. We also believe that this approach can help to overcome many of the problems of recording and reporting in follow up studies, so aptly described by Ruskin's elegant prose.[1]

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References

1. Ruskin J. *The stones of Venice.* Boston: Dana Estes, 1851: foreword.
2. Stanley F, Alberman E. Birthweight, gestational age and the cerebral palsies. In: Stanley F, Alberman E, eds. *The epidemiology of the cerebral palsy.* Clinics in developmental medicine No 87. London: Spastics International Medical Publications, 1984:57–68.
3. Ingram TTS. A historical review of the definition and classification of the Cerebral Palsies. In: Stanley F, Alberman E eds. *The epidemiology of the cerebral palsy.* Clinics in Developmental Medicine No 87. London: Spastics International Medical Publications, 1984:1–11.
4. Hagberg B, Hagberg G, Olow L. The changing panorama of cerebral palsy in Sweden, 1954–1970. II. Analysis of various syndromes. *Acta Paediatr Scand* 1975;**64**:193–200.
5. Bax M. Terminology and classification of cerebral palsy. *Dev Med Child Neurol* 1964;**6**:295–7.
6. Little WJ. On the influence of abnormal parturition, difficult labours, premature birth, and asphyxia neonatorum, on the mental and physical condition of the child, especially in relation to deformities. *Transactions of the Obstetrical Society of London* 1862;**3**:293–344. (Reprinted in *Cerebral Palsy Bulletin* 1958;**1**: 5–36.)
7. Hagberg B, Hagberg G, Olow L. The changing panorama of cerebral palsy in Sweden 1954–1970. I. Analysis of the general changes. *Acta Pediatr Scand* 1975;**64**:187–92.
8. Blair E, Stanley FJ. Interobserver agreement in the classification of cerebral palsy. *Dev Med Child Neurol* 1985;**27**:615–22.
9. Nelson KB, Ellenberg JH. Children who 'outgrow' cerebral palsy. *Pediatrics* 1982;**69**:529–35.
10. Nelson KB, Ellenberg JH. Neonatal signs as predictors of cerebral palsy. *Pediatrics* 1979;**64**:225–32.
11. Larroche JC, Bethmann O, Couchard M. Brain damage in the premature infant: early lesions and new aspects of sequelae. *Ital J Neurol Sci* 1986;**5**(suppl):43–52.
12. Costello AMdeL, Hamilton PA, Baudin J, *et al.* Prediction of neurodevelopmental impairment at 4 years from brain ultrasound appearance in very preterm infants. *Dev Med Child Neurol* 1988;**30**:711–22.
13. Amiel-Tison C, Dube R, Garel M, Jequier JC. Outcome at age 5 years of full term infants with transient neurologic abnormalities in the first year of life. In: Stern L, ed. *Intensive care IV.* New York: Masson, 1983:247–57.
14. Owen MJ, Lewis SW, Murray RM. Obstetric complications and schizophrenia: a computed tomographic study. *Psychol Med* 1988;**18**:331–9.
15. Hadders-Algra M, Huisjes HJ, Touwen BCL. Perinatal correlates of major and minor neurological dysfunction at school age: a multivariate analysis. *Dev Med Child Neurol* 1988;**30**:472–81.
16. Hadders-Algra M, Huisjes HJ, Touwen BCL. Perinatal risk factors and minor neurological dysfunction: significance for behaviour and school achievement at nine years. *Dev Med Child Neurol* 1988;**30**:482–91.
17. Stewart AL, Costello AMdeL, Hamilton PA, *et al.* Relation between neurodevelopmental status at one and four years in very preterm infants. *Dev Med Child Neurol* 1989 (in press.)
18. Prasse DP, Siewert JC, Ellison PH. McCarthy performance and neurological functioning in children born 'at risk'. *Journal of Psychoeducational Assessment* 1983;**1**:273–83.
19. Amiel-Tison C, Ellison P. Birth asphyxia in the fullterm newborn: early assessment and outcome. *Dev Med Child Neurol* 1986;**28**:671–82.
20. Amiel-Tison C, Grenier A. *Neurologic assessment within the first year of life.* New York: Oxford University Press, 1986.
21. Amiel-Tison C. Pediatric contribution to the present knowledge on the neurobehavioural status at birth. In: Mehler J, Fox R, eds. *Neonate cognition.* Hillsdale: Laurence Erlbaum, 1985: 265–280.
22. Thomas A, Saint-Anne Dargassies S. *Etudes neurolgiques sur le nouveau-né et le jeune nourrisson.* Paris: Masson, 1952.
23. Peiper A. *Cerebral function in infancy and childhood.* New York: Consultants Bureau Enterprises, 1963.
24. Chapelais JdeZ, McFarlane JA. A review of 404 'late walkers'. *Arch Dis Child* 1984;**59**:512–16.
25. Frostig M, Welty Lefever D, Whittlesey JRB. A developmental test of visual perception for evaluating normal and neurologically handicapped children. *Percept Mot Skills* 1961;**12**:383–94.
26. Saint-Anne Dargassises S. *The neuromotor and psychoaffective development of the infant.* Amsterdam: Elsevier, 1986.
27. Amiel-Tison C. Cerebral damage in full-term: aetiological factors, neonatal status and long term follow-up. *Biologia Neonatorum* 1969;**14**:234–50.
28. Stewart AL, Reynolds EOR, Hope PL, *et al.* Probability of neurodevelopmental disorders estimated from ultrasound appearance of brains of very preterm infants. *Dev Med Child Neurol* 1987;**29**:2–11.
29. Stewart AL, Hope PL, Hamilton PA, *et al.* Prediction in very preterm infants of satisfactory neurodevelopmental progress at 12 months. *Dev Med Child Neurol* 1988;**30**:53–63.
30. Grenier A. Prévention des déformations précoces de hanche chez les nouveau-nés à cerveau lésé. *Ann Pediatr* 1988;**35**:423–7.

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Regular review

Neonatal haemostasis

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The newborn baby's haemostatic mechanism is immature and does not develop fully until the age of 6 months. This does not seem to cause clinical problems for the healthy neonate, but may contribute to morbidity in the sick and premature infant.

Platelets

Platelets have been identified in the fetal circulation at 11 weeks' gestation,[1] and fetoscopy has shown their numbers to be within the adult range from 15 weeks' gestation onwards.[2] On the basis of this and other information[3] healthy infants, both premature and born at term, should be considered thrombocytopenic if they have platelet counts below $150\times10^9/l$, regardless of their period of gestation. Thrombocytopenia is, however, common among sick infants. A unique set of conditions surrounds the newborn infant causing an unusual susceptibility to thrombocytopenia—for example, maternal antibodies, congenitally acquired infections, and hypoxia. The incidence of thrombocytopenia has been variably reported. A prospective study of 807 consecutive infants admitted to a neonatal intensive care unit found platelet counts below $150\times10^9/l$ in 174 (22%) infants, and below $100\times10^9/l$ in 97 (12%).[4] In three quarters of the thrombocytopenic infants the lowest platelet count was on day 4 and the thrombocytopenia had resolved by day 10. Investigations suggested that increased consumption of platelets was the underlying mechanism. Only 21% of the markedly thrombocytopenic infants (platelet count $<100\times10^9/l$) had evidence of disseminated intravascular coagulation and 12% had had exchange transfusions. Of the risk factors assessed, birth asphyxia correlated most closely with the subsequent development of a low platelet count. It is difficult to assess the clinical impact of thrombocytopenia on the newborn because thrombocytopenic infants are usually sick, or premature, or both, and have a variety of medical complications that by themselves can contribute to morbidity and mortality. The same study reported that thrombocytopenic infants (platelet count $<100\times10^9/l$) weighing less than 1500 g at birth had three and a half times the risk of intraventricular haemorrhage of infants who were not thrombocytopenic, and who were matched for age, weight, and disease, even when infants with disseminated intravascular coagulation were excluded from analysis.[5]

Defective platelet aggregation in response to stimulation by adrenaline, collagen, and adenosine diphosphate has been reported in both premature infants and those born at term.[6] The response to adrenaline is particularly impaired, and this may be related to a deficiency of α-adrenergic receptor sites on the neonatal platelet membrane. In contrast, aggregation in response to ristocetin is increased and this may be explained by the raised concentration of von Willebrand factor in the newborn. Despite impaired platelet function, newborn babies have bleeding times that are normal or shorter than that of the adult.[5 7] This may be due to either raised concentrations of von Willebrand factor[5] or to reduced prostaglandin I_2-like regenerating activity,[8] both of which are present in the newborn. Although impaired platelet function does not cause a bleeding tendency, newborn babies are more susceptible to inhibition of platelet aggregation by aspirin and promethazine taken by the mothers.

Procoagulants

Clotting factors in the circulation of the newborn have been synthesised by the fetus and do not cross the placenta. Fetoscopy has provided valuable data about coagulation factors at various gestational ages. The information suggests that factors I,[9 10] V, and VIII[3] are within the normal adult range from the beginning of the third trimester onwards. All other factors are variably reduced at birth, being lower in the preterm infants, and dependent on the gestational age. Infants from 24 weeks' gestation onwards have mean fibrinogen concentrations within the adult range, suggesting that a fibrinogen concentration of 1·5 g/l at birth should be regarded as the lower limit of normal in both premature infants and those born at term.[9] [10] Fetal fibrinogen is comparatively insensitive to thrombin induced proteolysis and has an increased sialic acid content compared with adult fibrinogen; this varies with the degree of prematurity and correlates with the prolongation of the thrombin clotting time. Although the role of sialic acid in the conversion of fibrinogen to fibrin is controversial, its removal from the fibrinogen molecule enhances clot formation by thrombin. There is no firm evidence that fetal fibrinogen is comparable with fetal haemoglobin and coded for by a specific gene. Because similar abnormalities of fibrin formation are encountered in liver disease in adults, fetal fibrinogen may represent a postsynthetic modification of a basic fibrinogen protein in which hypersialation occurs in the fetal liver.

The procoagulants that are dependent on gestational age include the vitamin K dependent factors (factors II, VII, IX, and X), the contact factors (factors XI, XII, prekallikrein, and high molecular weight kininogen), and factor XIII.

At term the vitamin K dependent factors are about 50% of adult values, and at 24 weeks' gestation are about half that concentration.[11] Factor XIII concentrations are in a similar range at term. As only small quantities of factor XIII are required for clot stabilisation, the decreased activity in the newborn infant is unlikely to have any clinical relevance. Concentrations of contact factors have been reported to be 20–30%, and 30–50%, of adult values in preterm and term infants, respectively.

Anticoagulants

Four principal anticoagulants have been identified that act as inhibitors in different parts of the coagulation cascade. Antithrombin III, protein C, and protein S are dependent on gestational age; α_2 macroglobulin is not. Antithrombin III concentrations in the infant born at term are about 60% of the adult values, and further reduced in the preterm infant. Antithrombin III inhibits factors II and X. As concentrations parallel these vitamin K dependent coagulation proteins, a balance of procoagulants and anticoagulants exists.[11]

Protein C and protein S are vitamin K dependent factors and reduced to concentrations similar to those of other vitamin K dependent proteins. Protein S acts as a cofactor for protein C, which in its activated form inhibits activated factors V and VIII. Unlike protein C and protein S, factors V and VIII are not reduced at birth and consequently an imbalance in favour of thrombosis exists.[11] This physiological imbalance does not seem to lead to thrombosis. In the adult, protein S exists in a free active form (40%), and in an inactive form complexed to C4b-binding protein (60%). Newborn babies have low or undetectable concentrations of C4b-binding protein, which results in most of their protein S being in the free active form.[12] This comparatively high concentration of active protein S may enhance the potential of the protein C pathway, and consequently compensate in part for the low concentrations of other inhibitors.[12] α_2 Macroglobulin concentrations are raised above the adult range in both the term and preterm infant,[11] and likewise this inhibitor may have a protective role against thrombosis at a time when concentrations of the other principal anticoagulants are low.

Although plasminogen concentrations are about 50% of adults values at term, and do not reach adult concentrations until about 6 months of age, fibrinolytic activity seems to be increased in the newborn.[13]

A recent study of healthy infants born at term confirms that coagulation tests vary with the postnatal age, and that different coagulation factors show different postnatal patterns of maturation, but that most coagulation factors achieve near adult values by 6 months of age.[14]

Specific inherited coagulation defects

Although haemorrhage is usually due to an acquired coagulation defect in the newborn period, inherited coagulation defects may also present at this time and a family history is not always available. Isolated haemorrhage in an otherwise healthy infant is particularly suggestive, especially if the infant has been bottle fed. Factor deficiencies associated with neonatal haemorrhage include those of factors VIII, IX, XIII, VII, X, and afibrinogenaemia. It is generally only severely affected infants (<1%) who present at this age. Haemophilia A and B and von Willebrand's disease account for more than 90% of inherited clotting factor deficiencies, and between 5–10% of affected infants will present in the neonatal period.

Thrombosis

The peak incidence of thrombosis in the paediatric age group is in the neonatal period, when it is most frequently iatrogenic and associated with indwelling catheters, although not exclusively so.[15] There is no real evidence that the physiological reduction of anticoagulants in the newborn contributes to thrombosis. Although infants have plasma concentrations of antithrombin III and protein C (which would be associated with thrombotic episodes in adults), even in congenital antithrombin III and protein C deficiency thrombosis is exceptional in childhood. Other physiological changes may compensate for the low concentrations of these inhibitors. Raised values of the other inhibitors, in particular α_2 macroglobulin, free active protein S, C1- esterase inhibitor, and heparin cofactor II, have been proposed. A profound reduction of either antithrombin III or protein C, however, does cause disease. Newborn infants with homozygous protein C deficiency have been reported with fatal fulminant thrombosis.[16-18] A small number of infants born to mothers with congenital antithrombin III deficiency have died of thrombotic complications in the neonatal period.[19 20] Congenital antithrombin III deficiency was suspected but not verified by assay.

Homozygous antithrombin III and protein C deficiencies are probably incompatible with life. Infants born to mothers with antithrombin III deficiency should have their antithrombin III concentrations measured at birth. It has been advocated that affected infants should receive prophylactic antithrombin III concentrate to maintain values appropriate for the newborn until the infant's own concentration has risen to about 50%.[21] The risk of viral transmission by the blood product, however, must be balanced against the risk of thrombosis and a more conservative approach may be indicated. A few cases of homozygous protein C deficiency have been diagnosed early and treatment instigated. Intitial treatment should always be with fresh frozen plasma, with or without anticoagulation. The half life of protein C is about seven hours, and to maintain adequate concentrations treatment would have to be given at such frequent intervals that it is not a practical long term solution. Factor IX concentrate has been used successfully for administering protein C.[22] The factor IX concentrate should be selected with care to minimise its thrombotic risk.[23] Infusions of 50–75 U/kg of protein C every other day seem sufficient to stop thrombotic episodes, although conentrations approach zero by the end of 48 hours. Warfarin has also been used with some success. It is difficult, however, to achieve a stable therapeutic dose in the newborn, and this treatment does nothing to raise the concentration of protein C or correct the imbalance between protein C and its procoagulants.

Haemorrhagic disease of the newborn

Vitamin K_1 is safe and virtually no infant who is given it develops classic haemorrhagic disease of the newborn.[24] McNinch *et al* reported an incidence of local recurrence of haemorrhagic disease of the newborn of 1:1200 live births in an area in which prophylactic vitamin K_1 was not given routinely.[25] All infants were breast fed and had not received vitamin K_1 at birth. Three of the infants died. This highlights the need for vitamin K prophylaxis for all newborn babies, especially in a society in which breast feeding is becoming increasingly popular. Oral administration seems an attractive alternative to the parenteral route. There is little information on the intestinal absorption of vitamin K in the newborn, but one study that compared oral with parenteral administration of 1 mg vitamin K_1 reported that neonates receiving oral vitamin K_1 achieved median peak plasma concentrations of 73 ng/ml at four hours compared with 1781 ng/ml at 12 hours with parenteral administration.[26] Although the concentration after oral administration was considerably lower than that after parenteral administration, it was still some 300 times the adult median, and nearly 4000 times the estimated maximum cord plasma concentration. No infant in the study developed haemorrhagic disease of the newborn. It is clear that vitamin K prophylaxis should be given to all newborn infants.

Should infants who are exclusively breast fed receive further vitamin K supplementation after the immediate neonatal period? Late haemorrhagic disease of the newborn has been reported in a small number of breast fed infants who had received vitamin K at birth. This, however, seems to be a rare finding. At particular risk are breast fed infants who develop diarrhoea and infants who are treated with antibiotics. The American Academy of Pediatrics stated in 1979 'Breast-fed infants who develop diarrhea of longer than several days duration should be given one intramuscular injection of vitamin K (1·0 mg). Infants at continued risk of vitamin K deficiency (eg, malabsorption, biliary atresia) should be given supplements of vitamin K at regular intervals'.[27]

Conclusion

Newborn infants are at particular risk of both thrombotic and haemorrhagic problems. These conditions are under investigated because of the difficulty of obtaining adequate volumes of blood for analysis, and this often means that the infants are inappropriately treated. It is important that a laboratory serving a neonatal unit establishes specialised standardised microtechniques that are reproducible with respect to the reagents, methods, and volumes used for routine coagulation screening.

References

[1] Bleyer WA, Hakami N, Shepard TH. The development of hemostasis in the human fetus and newborn infant. *J Pediatr* 1971;**79**:838–53.

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Current topic

Pulmonary vascular air embolism in the newborn

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Pulmonary vascular air embolism is a rare, and almost invariably fatal, complication of positive pressure ventilation of newborn infants. There have only been 50 cases described in the world literature to date.[1–16] The rarity of the condition and the clustering of some cases, which may be related to specific local factors, do not allow a meaningful calculation of incidence.

Characteristics of reported cases

Adequate numbers of cases have been reported to develop a profile of those infants susceptible to pulmonary vascular air embolism. The reported characteristics of these infants, and three additional unreported cases of our own, are summarised in table 1. Affected infants are usually premature with a mean gestational age of 30 weeks, and a mean birth weight of 1328 g. The average postnatal age at onset was the third day of life. This, with a male:female ratio of 2·1 (33:16) in those cases where sex was documented, reflects the 91% incidence of the respiratory distress syndrome of the newborn in this population. The remaining 9% of cases were term infants with various diagnoses including meconium aspiration, viral pneumonia, amniotic fluid aspiration, and congenital alveolar dysplasia.

Table 1 *Characteristics of the population of infants reported to have developed pulmonary vascular air embolism. (No=the number of reported cases for which each characteristic was documented)*

	No of cases	*Mean (SD)*	*Range*
Gestational age (weeks)	47	29·7 (4·2)	24–40
Birth weight (g)	53	1328 (590)	670–3110
Age at occurrence (hours)	47	63 (91)	3–456
Fractional inspiratory oxygen	27	0·9 (0·1)	0·5–1·0
Peak inspiratory ventilator pressure (cm H_2O)	45	40 (17)	20–90
Peak expiratory ventilator pressure (cm H_2O)	38	7 (6)	0–40

Unreported cases (SK Lee and AK Tanswell): case 1, boy of 25 weeks' gestation weighing 850 g; peak inspiratory/peak expiratory ventilator pressure (PIP/PEP) was 36/5 cm H_2O; he had pulmonary interstitial emphysema and arrhythmia. Case 2, girl of 27 weeks' gestation weighing 880 g; PIP/PEP was 34/4 cm H_2O; she had pulmonary interstitial emphysema, pneumothorax, and arrhythmia. Case 3, boy of 24 weeks' gestation weighing 640 g; PIP/PEP was 18/4 cm H_2O; he had pulmonary interstitial emphysema and arrhythmia.

As would be expected all infants had severe pulmonary insufficiency, as evidenced by average peak inspiratory/expiratory ventilator pressures (PIP/PEP) of 41/7 cm H_2O with a peak fractional inspiratory oxygen of 0·9. Among all the cases reported there is no correlation between PIP/PEP and gestational age, or with time of occurrence. The time of occurrence of pulmonary vascular air embolism does, however, correlate with gestational age (n=41; linear regression equation: y=9·6x−228·98; r=0·464; p<0·01) for the total populations in which this was reported. The most significant correlations were observed when only data for premature infants of <37 weeks' gestation (n=37; r=0·593; p<0·001), or <2500 g birth weight (n=45; r=0·552; p<0·001) were included in the regression analyses. The four reported infants with birth weights of >2449 g had a mean (SD) time of occurrence of 113 (92) hours, while the four reported infants with a gestational age of >36 weeks' gestation had a mean time of occurrence of 69 (38) hours.

Clinical signs

Diagnosis is usually made from a radiograph ordered for suspected air leak, but there are associated phenomena reported for these infants (table 2) which may suggest the diagnosis. There was an overall incidence of air leak syndromes, other than pulmonary vascular air embolism, of 94%. The presenting signs of pulmonary vascular air embolism were usually sudden and dramatic. The

Table 2 *Associated phenomena in infants who develop pulmonary vascular air embolism. The % values shown are the totals derived from the collected literature in which clinical events were reported*

Phenomenon	%
Air leak syndrome	94
Interstitial emphysema	63
Pneumothorax	36
Pneumomediastinum	24
Pneumopericardium	14
Arrhythmia/abnormal electrocardiogram	49
Catheter air	40
Circulatory collapse	35
Cutaneous signs	11

most common signs included sudden collapse with either pallor or cyanosis, hypotension, bizarre electrocardiogram irregularities varying from tachycardia to bradycardia, with the latter being more common. A millwheel murmur was heard in several cases and the heart sounds were also noted to be distant and diminished. Blanching and migrating areas of cutaneous pallor were noted in several cases and, in one of our own cases we noted bright pink vessels against a generally cyanosed cutaneous background. This we attributed to direct oxygenation of erythrocytes adjacent to free air in the vascular system, while the tissues continued to be poorly perfused and oxygenated. The most distinctive sign of pulmonary vascular air embolism, observed in half of the reported cases, is the finding of free air when blood is withdrawn from the umbilical arterial catheter. Columns of air, or a frothy mixture of blood and air, were often obtained. Another related phenomenon, observed in two reported patients[16] and in one of our infants, was an inappropriate high arterial oxygen concentration recorded from intra-aortic oxygen electrodes in direct contact with gas bubbles. This was also observed using a transcutaneous oxygen monitor in one case.[16] A radiograph is diagnostic, and free air may be seen in both the arterial and venous systems, as well as in the heart (figure). In 75% of reported cases the radiographs were taken antemortem. Postmortem radiographs need to be interpreted with caution as intravascular air may appear as early as 25 minutes after death.[3]

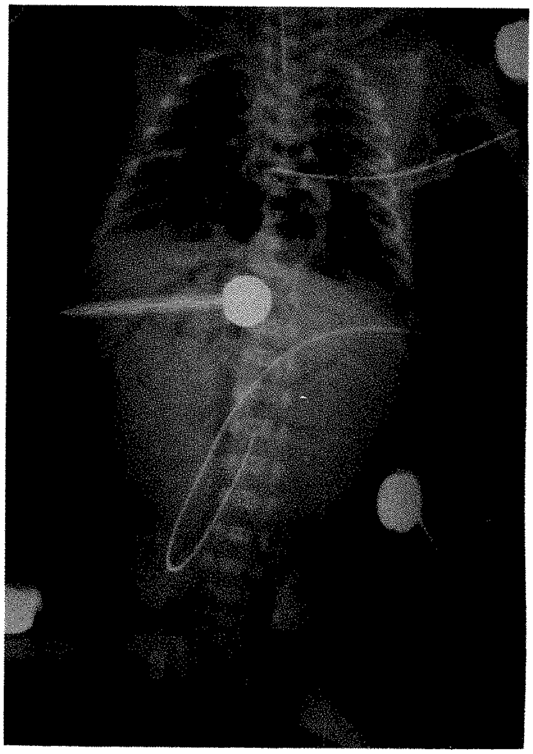

Figure *Anteroposterior chest and abdominal radiograph of a case of pulmonary vascular air embolism, showing pulmonary interstitial emphysema with a left thoracotomy tube, intracardiac air, and air in the major vessels of the neck, chest, and abdomen.*

The typical case of pulmonary vascular air embolism will be of very low birth weight and have respiratory distress syndrome, requiring very high ventilation pressures, with an existing air leak. The embolism will usually occur in the first week of life and, in most cases, there will be unusual phenomena which should alert the physician to the possibility of this rare occurrence. A bizarre pattern on electrocardiography, the presence of catheter air, a millwheel murmur, migrating pallor in small vessels, and acute massive fluctuations of continuously monitored oxygen tension are all suggestive, and justify immediate aspiration through an umbilical arterial catheter if present.

Mortality

Only four of the 53 infants in this review survived the immediate event. One infant died from a recurrence 16 days after the first episode,[9] while another died from pneumonia 13 days after surviving pulmonary vascular air embolism.[15] Of the two long term survivors, the case described by Kogutt[6] had asymptomatic pulmonary vascular air embolism shown by a routine radiograph. The other is one of our own cases who, despite a myocardial infarction as a result of the pulmonary vascular air embolism, survived until 7 months of age when he died from respiratory failure due to viral pneumonia superimposed on chronic bronchopulmonary dysplasia.

Pathogenesis

Gregory and Tooley postulated that air embolism occurred as a consequence of air being injected into pulmonary veins by mechanical ventilation.[1] A potential portal of entry into the pulmonary interstitium had been previously shown by Macklin and Macklin using gelatin varmine particle techniques to show microscopic alveolar rupture in pulmonary interstitial emphysema.[17] Further extension of free air into the capillary bed was shown by Lenaghan *et al* with the demonstration of air embolism in mechanically ventilated dogs with pulmonary interstitial emphysema.[18] He observed fistularisation distal to the terminal bronchiole which occurred at lower pressures in shocked lungs, and was not ameliorated by the use of prophylactic chest tubes. Bowen *et al* were finally able to demonstrate a direct communication between the airway, the interstitium, and small vascular channels with barium studies at autopsy of a human infant who died from pulmonary vascular air embolism. There are a number of reasons why gas is found in both the arterial and venous systems, including retrograde flow into the right heart through an incompetent pulmonary valve,[18] and passive retrograde flow of gas bubbles because of their buoyancy.[8]

While there seems to be a relationship between pressure applied and the type of air leak observed in dogs, with successively higher pressures required for pneumothorax/pneumomediastinum, pneumoperitoneum, and air embolism,[19] we were unable to identify any specific threshold levels for pulmonary vascular air embolism from the analysis of the data provided by the reports in the literature. Air leak syndromes, however, did precede pulmonary vascular air embolism in 94% of the reported cases, and its development would therefore seem to require a greater pressure than the other air leak syndromes. The observed correlation of the timing of pulmonary vascular air embolism with gestational age or birth weight, but no relationship with inflation pressure, suggests that barotrauma is inflicted earlier in the more immature lung, and that the development of pulmonary vascular air embolism is determined as much by the physical characteristics of the lung being inflated as by the characteristics of the inflation. The mature infants with pulmonary vascular air embolism had a time of onset somewhat earlier than would be predicted from the regression line developed for premature infants. It is possible that these infants had an inherent predisposition to air leak due to abnormal tissue elastance, though this is only speculation in the absence of appropriate histological evaluation.

Trauma to the lung may have a more significant part to play in the development of pulmonary vascular air embolism than has been generally appreciated. In two of our three cases there was evidence of trauma related to the introduction of chest tubes. Lung perforation occurs in 25–30% of infants with respiratory distress syndrome who have chest tubes inserted for drainage of pneumothoraces.[20] Laceration of lung tissue is reported to favour reversal of the intra-bronchial pressure—pulmonary venous pressure gradient thereby increasing the risk of pulmonary vascular air embolism.[21]

Cardiac arrhythmia is a common presenting sign of pulmonary vascular air embolism, which may be due both to the effects of air embolism on the heart and on the brain. Studies in cats suggest that arrhythmias produced by cerebral air embolism can be abolished by sympathectomy.[22]

The prognosis for pulmonary vascular air embolism remains poor, and the neurologic outcome for survivors is unclear. Injection of air into the carotid artery of adult gerbils rapidly results in multifocal brain lesions.[23] Studies in dogs suggest that outcome may be better if the embolised gas is oxygen rather than air,[24] as might be expected from its better solubility. Many of the human infants that develop this complication of ventilatory treatment will already be receiving 100% oxygen, but when this is not the case a change to 100% oxygen might have some protective effect.

Prevention and treatment

Our analysis does not allow us to establish clear guidelines for prevention. Avoidance of high airway pressures would be advantageous, though these are rarely applied if there is an alternative. High frequency ventilation may eventually offer such an alternative, while the use of surfactant applied down the airways may reduce the need for extremely high ventilation pressures. In those infants who have pneumothorax it may be possible to reduce the incidence of pulmonary vascular air embolism by using soft rubber catheters, instead of stiff plastic chest tubes, for drainage as these may be less traumatic to the surface of the lung.

Where pulmonary vascular air embolism has occurred, early withdrawal of air from the umbilical artery catheter may be of benefit, especially if the air leak is small and self sealing, or the air has been introduced through an intravascular line. In this situation it may be worthwhile attempting to minimise neurological damage by using 100% oxygen and placing the infant in the Trendelenberg and left posterior-anterior position.[4]

The incidence of pulmonary vascular air embolism is likely to increase with the improved survival of very low birthweight infants, and the incidence may already be higher than we appreciate, because of a lack of recognition of the acute event, and the rapid loss of signs after death.[11] The prognosis for the condition remains poor, but may improve with greater recognition allowing earlier aggressive management.

References

[1] Gregory GA, Tooley WH. Gas embolism in hyaline membrane disease. *N Engl J Med* 1980;**282:**1141–2.

[2] Bowen FW, Chandra R, Avery GB. Pulmonary interstitial emphysema with gas embolism in hyaline membrane disease. *Am J Dis Child* 1973;**126:**117–8.

[3] Quisling RG, Poznanski AK, Roloff DW, Borer RC. Postmortem gas accumulation in premature infants. *Radiology* 1974;**133:**155–9.

[4] Shook DR, Cram KB, Williams HJ. Pulmonary venous air embolism in hyaline membrane disease. *American Journal of Radiology* 1975;**125:**538–42.

[5] Rodriguez C, Eschavarria IJL, Portilla AJ. Embolia gaseosa masiva en un recien nacido tratado con respirador de presion positiva. *Boletinmedico del Hospital Infantil de Mexico* 1977;**34:**349–56.

[6] Kogutt MS. Systemic air embolism secondary to respiratory therapy in the neonate: six cases including one survivor. *Journal of Roentgenology* 1978;**131:**425–9.

[7] Opperman HC, Wille L, Obladen M, Richter E. Systemic air embolism in respiratory distress syndrome of the newborn. *Pediatr Radiol* 1979;**8:**139–45.

[8] Mahmud F. Air embolism from mechanical ventilation in respiratory distress syndrome. *South Med J* 1979;**72:**783–7.

[9] Cordello H, Fodor M. Massive luftembolie als komplication kontrollierter beatmung. *Klin Padiatr* 1979;**191:**339–43.

[10] Baur MN, Otten A, Briner J. Plotzlicher tod durch massive luftembolie bei respirator-beatmug im neugeborenanalter. *Helv Paediat Acta* 1979;**34:**147–53.

[11] Rudd PT, Wigglesworth JS. Oxygen embolus during mechanical ventilation with disappearance of signs after death. *Arch Dis Child* 1982;**57:**237–9.

[12] Fraudenberg V, Eberle P. Die luftembolie als seltenes ereignis des air-leakage-komplexes beim beatmeten fruhgeborenen. *Monatsschr Kinderheilkd* 1984;**132:**913–4.

[13] Gortner L, Pohlandt F. Zentral-venose luftembolie bei einem beatmeten frugheborenen mit atemnotsyndom. *Klin Padiatr* 1984;**196:**385–7.

[14] Toischer HP, Gortner L. Systemische luftembolie unter repiratortherapie des neugeborenen. *Fortschritte auf dem Gebiete der Rontgenstrahlen* 1984;**141:**414–7.

[15] Weiner JH, Kliegmen RM, Fanaroff AA, Waldemar AC. Pulmonary venous air embolism in the neonate. *Crit Care Med* 1986;**14:**67–9.

[16] Fenton TR, Bennett S, McIntosh N. Air embolism in ventilated very low birthweight infants. *Arch Dis Child* 1988;**63:**541–9.

[17] Macklin MT, Macklin CC. Malignant interstitial emphysema of the lungs and mediastinum as an important occult complication in many respiratory diseases and other conditions. *Medicine* 1944;**23:**281.

[18] Leneghan R, Silva YJ, Walt AJ. Hemodynamic alterations associated with expansion rupture of the lung. *Arch Surg* 1969;**99:**339–43.

[19] Grosfield JL, Boger D, Clatsworthy HW. Hemodynamic and manometric observations in experimental air block syndrome. *J Pediatr Surg* 1971;**6:**339–34.

[20] Moessinger AC, Driscoll JM, Wigger HJ. High incidence of lung perforation by chest tube in neonatal pneumothorax. *J Pediatr* 1978;**92:**635–7.

[21] Chiu CJ, Golding MR, Linder JB, Fries CC. Pulmonary venous air embolism: a hemodynamic reappraisal. *Surgery* 1967;**61:**816–9.

[22] Evans DE, Kobrine AI, Weathersby PK, Bradley ME. Cardiovascular effects of cerebral air embolism. *Stroke* 1981;**12:** 338–44.

[23] Garcia JH, Klatzo I, Archer T, Lossinsky AS. Arterial air embolism: structural effects on the gerbil brain. *Stroke* 1981;**12:** 414–21.

[24] Fries CC, Levoweitz B, Adler S, *et al.* Experimental cerebral gas embolism. *Ann Surg* 1957;**145:**461.

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ook reviews

tal and Neonatal Neurology and urosurgery. Edited by M Levene, M nnett, and J Punt. Pp 618: £95 hardback. urchill Livingstone, 1988. ISBN 0–443– 713–2.

is is a marvellous book. Professor vene (neonatologist), Professor Bennett stetrician), and Mr Jonathan Punt urosurgeon) have managed to compile latest views of 55 international experts all aspects of fetal and neonatal rology. These are presented clearly in a ll laid out, well referenced, and thetically pleasing large book.

would emphasise that the book is ally comprehensive from ultrasound of developing fetal nervous system to raventricular haemorrhage in the term; from assessment of prenatal hyxia to management and outcome of th asphyxia; from inborn errors of tabolism presenting neurologically to al and bacterial infections of the brain.

t is impossible to comment on all 48 pters, but several are worthy of special ntion. Professor Prechtl (Groningen) l Dr L Dubowitz (Hammersmith) take succinctly through fetal and neonatal rological evaluation. Later on Professor nholz (Chicago) demonstrates, with h quality ultrasound, normal and some ormal development of the nervous em in the fetus. The next chapter by Dr (Bologna) and Professor Hobbins le) presents more superb pictures on er pathologies with good illustration of nesis of the corpus callosum.

nvestigative techniques—electroence- alography, evoked responses, magnetic onance imaging, and magnetic resonance ctroscopy—are summarised by res- tive experts in the field.

predictably strong section concerns natal, intrapartum, and peripartum as- xia. Professor Kjellmar from Gothen- g and Malcolm Levene cannot be more to date on this most important problem l although presenting no solutions, at t provide the reader with a balanced w of what can be done. Finally, the pter on seizures (Dr J Brown) and romuscular disorders (Dr J Heckmatt) of considerable clinical value.

have never read a book which combined well the physiology of the subject while retaining the clinical message. The content is all 1988 vintage and not one chapter is padding, the standard material obtainable in a general neonatal text book. The price is high so the market will be departmental libraries. I am sure this book will be very highly acclaimed.

J G Bissenden
CONSULTANT PAEDIATRICIAN
Dudley Road Hospital, Birmingham

Development of the Human Fetal Brain. An Anatomical Atlas. By Alison Feess-Higgins and Jeanne-Claudie Larroche. Pp 200: Frs 350 hardback. Masson, Paris, 1988. ISBN 2–85598–337.

This atlas covers the development of the fetal brain from 10 weeks' gestation to term. Text and legends to figures are in French and English throughout. Source material was selected from an anatomical collection compiled in the Centre de Recherches de Biologie du Développment in Paris. It is illustrated by black and white photographs and low power photomicrographs that are accompanied by annotated diagrams. Illustrations of the developing brain at three to four week intervals are grouped to show all the external surfaces and sets of whole sections through the brain in frontal, sagittal, and horizontal planes. Gestation and plane of section are clearly indicated at the top of each page. Legends set out briefly gestation, length of survival, body weight, and dimensions as well as dimensions of the brain. All this is preceded by a double page spread of the medial and lateral surfaces of the brain at two to four week intervals for rapid reference.

This is a splendid work, and its appearance is timely. It is not just the pathologist who will benefit from this atlas. The range and availability of imaging techniques for examination of the brain has increased enormously during the last 10 years. This book will be of enormous practical value to those involved with cerebral imaging both prenatally and in the neonatal nursery and is thus an essential reference work for any department undertaking such work. Its price is modest and I think it deserves a place on the bookshelf of any clinician concerned with cerebral function in the newborn.

J W Keeling
CONSULTANT PAEDIATRIC PATHOLOGIST
John Radcliffe Hospital,
Oxford

Fetal and Neonatal Growth. Vol 5. Edited by Forrester Cockburn. Pp 211: £37·50 hardback. John Wiley, 1988. ISBN 0–471–91679–X.

Guest editor Forrester Cockburn has drawn contributions from a host of international authorities in the field of fetal and neonatal growth to form volume five in this current series of publications on perinatal practice. The preface describes each volume as being a series of reviews of topical issues. Pursuant to the belief held by the late Professor Alan Moncrieff, that the study of growth and development was the basic science of paediatrics, this particular volume deserves the special attention of all those concerned with paediatric care. The volume consists of 10 chapters of variable length and detail.

A 'gentle' but informative first chapter introduces the genetics of fetal and postnatal growth and highlights the highly complex interaction between heredity and environment that modulate growth. The equally complex hormonal influences upon growth are comprehensively described in the following two chapters, including the role of epidermal growth factors, insulin like growth factors, thyroxine, and growth hormone. Methods of studying growth in the fetal and neonatal period are considered in the next two chapters, with reference to the experimental manipulation of placental growth and development, and the use of Doppler blood flow techniques in the study of fetal growth retardation. Both the principals and practise of this latter technique are described in detail. Excellent sections on the nutritional aspects of fetal and neonatal growth, and hepatic bone enzymes follow before growth factors occupy two further chapters. The final contribution on growth factors, including milk growth factors, produces an excellent bibliography for those interested in this burgeonning area of research. The penultimate chapter is an extensive and excellent contribution on the adaptation of the fetus to extrauterine nutrition, which should be required reading for students of human biology as well as clinicians. Finally, consideration is given to growth in the first year, with particular emphasis on the endocrine milieu rather than the auxological aspect.

The organisation of the material within this volume could have been improved upon; the five chapters concerned with growth factors from conception to the end of the first year of life could have been consecutive.

Readers interested in the relationship between early growth and long term growth will be disappointed and this aspect possibly warranted a short chapter, but nevertheless this is a useful, informative, and readable collection of papers.

P S W Davies
STAFF SCIENTIST
Clinical Infant Nutrition Group,
MRC Dunn Nutrition Unit, Cambridge

Neonatal Anesthesia. Edited by D Cook and J Marcy. Pp 262: £40 hardback. Appleton Davies Inc, 1988. ISBN 0-941022-10-2.

More than half this book on neonatal anaesthesia concentrates on the basic sciences, physiology, and pharmacology, and has the high standard of writing usual in American textbooks. It would prove a useful reference to both paediatric anaesthetists and neonatal paediatricians in training.

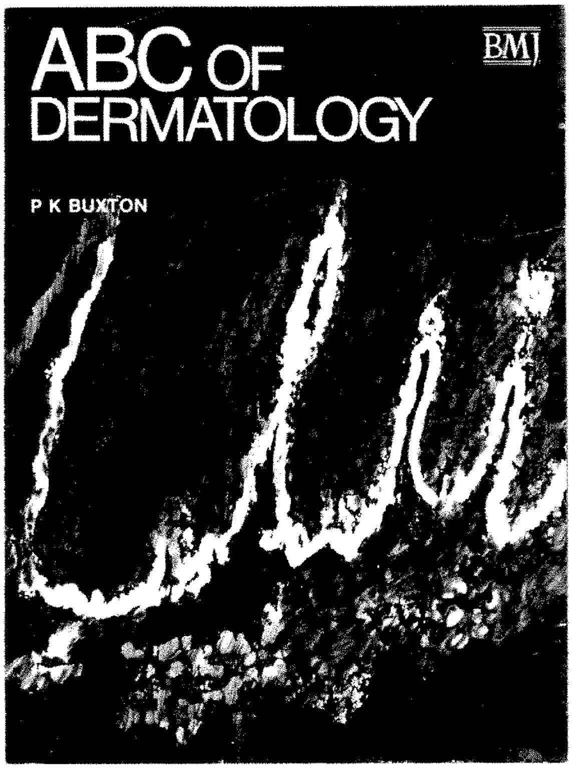
The chapters on anaesthetic technique and respiratory care, however, are less comprehensive and not entirely specifi
the neonatal age group, frequently re
ring to much older children.

A specific discussion on the rela
differences between a newly born baby
a chronologically older ex-premat
infant of similar gestational age seems
important omission, as does a section
the use of local anaesthetic agents, both
surgery and postoperative analge
though this probably reflects diffe
clinical practices across the Atlantic.

The editors state that they wished
avoid the cook book approach and w
doing this they have lost some of
usefulness because of omissions to trai
paediatric anaesthetists.

This book would provide a useful a
tion to the library shelf, but because of
scope and price would be unlikely to b
'best buy' for individuals.

A Macke
CONSULTANT ANAESTHE
Hospital for Sick Children, Lon



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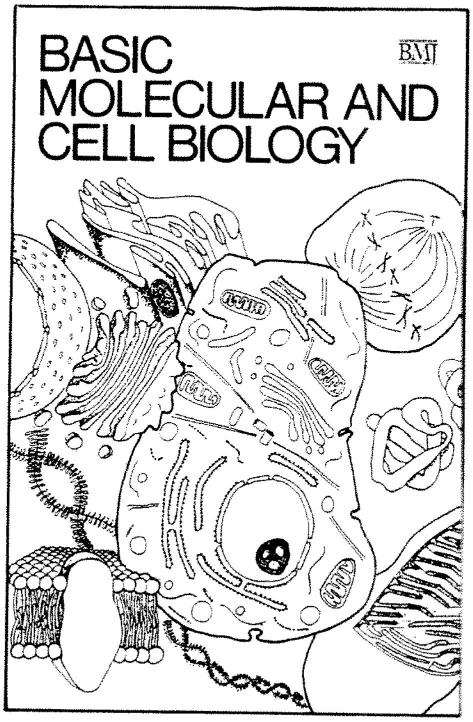

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The Journal of the British Paediatric Association

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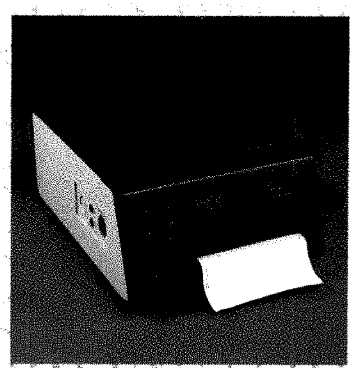

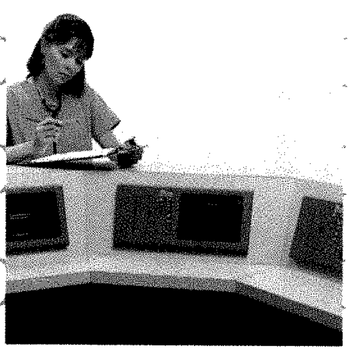

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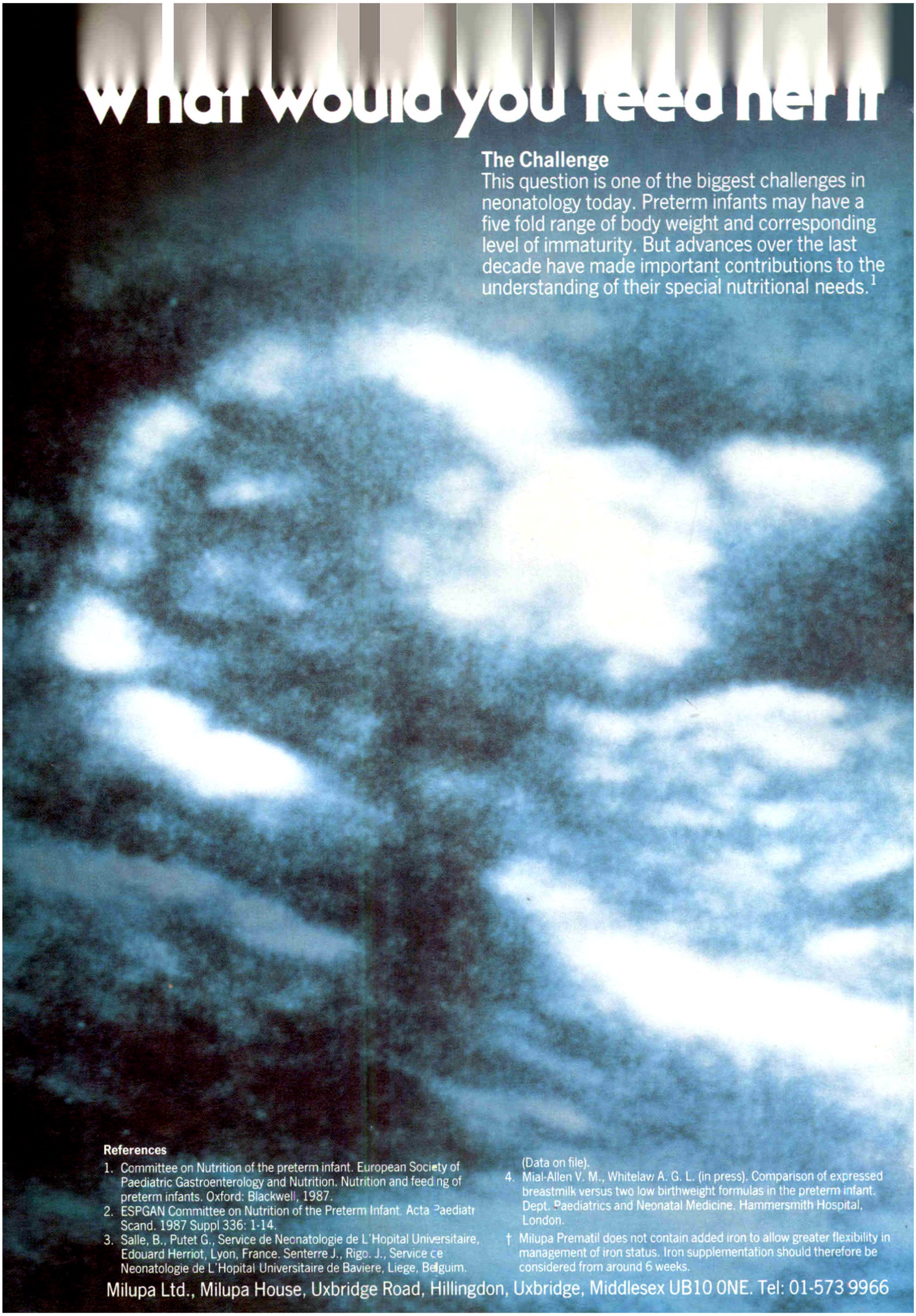

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References

1. Committee on Nutrition of the preterm infant. European Society of Paediatric Gastroenterology and Nutrition. Nutrition and feeding of preterm infants. Oxford: Blackwell, 1987.
2. ESPGAN Committee on Nutrition of the Preterm Infant. Acta Paediatr Scand. 1987 Suppl 336: 1-14.
3. Salle, B., Putet G., Service de Neonatologie de L'Hopital Universitaire, Edouard Herriot, Lyon, France. Senterre J., Rigo. J., Service de Neonatologie de L'Hopital Universitaire de Baviere, Liege, Belguim. (Data on file).
4. Mial-Allen V. M., Whitelaw A. G. L. (in press). Comparison of expressed breastmilk versus two low birthweight formulas in the preterm infant. Dept. Paediatrics and Neonatal Medicine. Hammersmith Hospital, London.

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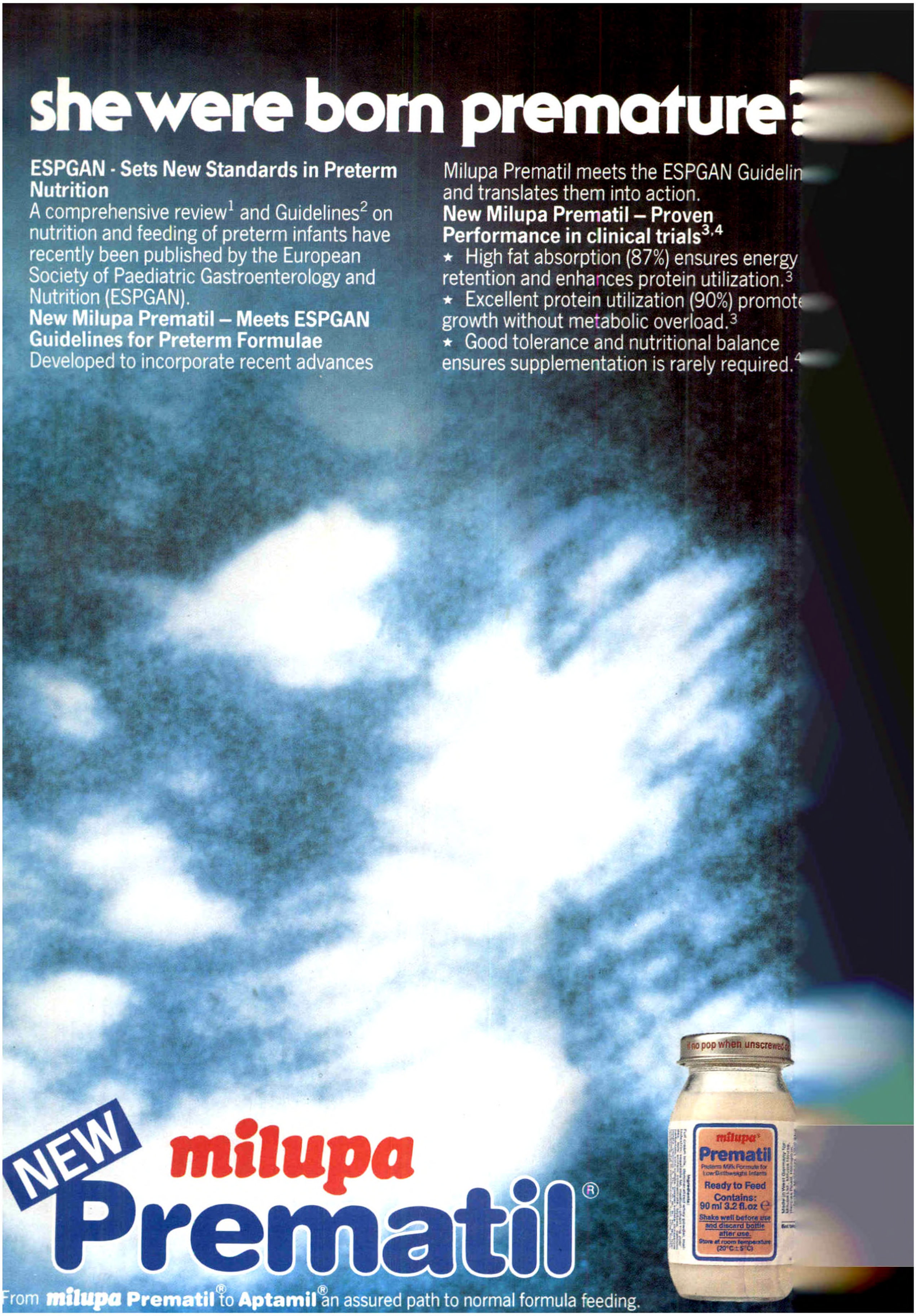

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Cerebral blood flow velocity variability after cardiovascular support in premature babies

J M RENNIE

Department of Paediatrics, University of Cambridge

SUMMARY The variability of cerebral blood flow velocity was measured in 20 very low birthweight infants. The area under the curve of the Doppler frequency spectrum was used and was obtained from the anterior cerebral artery in the infants before and after infusions of plasma or dopamine. Both interventions significantly reduced the variability without a demonstrable effect on cardiac output, heart rate, or cerebral blood flow velocity. The reduction was from a median of 13% to 5% after plasma and from 13% to 4% after dopamine. The effect was probably due to an increase in circulating blood volume. It is proposed that appreciable variations in systolic blood pressure occurring in phase with respiration indicate a low central venous pressure in infants, as in adults and animals. The variations could be transmitted to the cerebral circulation in the absence of autoregulation, and their detection in either the aorta or a cerebral artery may prove a useful index of hypovolaemia.

The theory that most instances of neonatal brain injury occur after alterations in cerebral blood flow remains unchallenged. Evidence continues to accumulate showing that the cerebral circulation of some preterm babies is 'pressure passive', easily transmitting variations of systemic blood pressure (F Cowan, M Eriksen, D Wertheim. Assessment of cerebral autoregulation in the preterm infant. Presented at the Neonatal Society, London, 1988).[1] High peaks in systemic blood pressure, low mean arterial pressure, and high beat to beat variability in systemic and cerebral blood pressure have all been suggested as risk factors preceding cerebral abnormalities.[2-4] Ultrasound can accurately diagnose many neonatal intracranial lesions and the information used to predict later neurodevelopmental handicap with improving sensitivity and specificity.[5] In addition to producing a real time image ultrasound can be used with the application of the Doppler principle to obtain information regarding the velocity of blood flow in vessels. While repeated accurate quantitation of cerebral blood flow remains impossible, work in which the limitations of the method have been understood has led to some useful observations regarding the cerebral circulation. One example is a high variability in some babies, the origins of which probably lie in cardiovascular changes secondary to respiration.[4]

Small changes in left ventricular stroke volume occur throughout the normal respiratory cycle, with a slight decrease in systolic blood pressure during the early phase of inspiration.[6] This finding when exaggerated is well known as pulsus paradoxus. Reversed pulsus paradoxus, with a rise in systemic blood pressure during positive pressure inflation,[7] is thought to result from an increase in left ventricular filling due to blood being 'squeezed' out of the lungs by the ventilator pressure.[8] Changes from respiration induced alterations in intrathoracic pressure probably make a major contribution to the blood pressure and cerebral blood flow velocity perturbations. The changes will be more variable in awake ventilated infants who may interact with positive pressure ventilation in several ways.[9] It is not surprising, therefore, that variability of cerebral blood flow velocity can be reduced by temporary cessation of artificial ventilation,[10] paralysis,[11] or inducing the baby to breathe in synchrony with his ventilator.[12] Spectral analysis of blood pressure and variations in cerebral blood flow velocity has shown a low frequency component with a periodicity identical to that of the respiratory cycle.[13]

Blood pressure variation during positive pressure respiration has been studied in ventilated adults and in animals; in both the variations are a sensitive indicator of hypovolaemia.[14] In order to investigate this possibility in preterm infants, cerebral blood flow velocity variability was measured in preterm babies before and after infusions of plasma and dopamine.

Subjects and methods

Twenty babies of median birth weight 1230 g (range from 713 to 1900) and median gestational age 29 weeks (range 24 to 32) who were treated with plasma (n=11) or dopamine (n=9) in the first 24 hours of life were studied. The protocol was approved by the local ethical committee, and the work carried out in the Cambridge neonatal intensive care unit. All the babies were ventilated for hyaline membrane disease, and the reason for treatment was usually a blood pressure below that usual for their weight,[15] although some babies received plasma for underperfusion of one or both lower limbs after insertion of an umbilical artery catheter without a low mean arterial pressure. Babies with low blood pressure were treated with plasma initially, and if this failed to improve the mean systolic pressure to about 40 mm Hg dopamine was instituted. No baby was studied twice and those in the dopamine group who received dopamine after plasma were studied at least one hour after colloid infusion.

Measurements of cerebral blood flow velocity were made from the anterior cerebral artery via the fontanelle using Duplex Doppler ultrasound (ATL Mk600). The Doppler shifted spectrum was collected by an Apple IIe microcomputer in order to calculate the time averaged mean velocity from 3·5 seconds of information.[16] The same software was used to collect spectra and calculate velocity from the Doppler shift obtained from blood flowing in the ascending aorta by using the real time image obtained via a suprasternal approach.[17] The diameter of the aorta was measured in a parasternal long axis view using M mode echocardiography enabling calculation of the cardiac output from the relation: flow=velocity×area.

Variability of cerebral blood flow was quantified by obtaining a paper record of the Fourier transform of the Doppler spectra and tracing the area under the curve for each of 10 consecutive cardiac cycles with computer assisted planimetry. Variability was then expressed as the coefficient of variation for the area, that is the standard deviation divided by the mean expressed as a percentage.

Estimates of cardiac output, anterior cerebral artery velocity and variability were made before and 30 minutes after either giving 10 ml/kg of plasma over 30 minutes or the start of a dopamine infusion at a dose of 10 µg/kg/minute. The observations described were made and the data stored on disc or on paper traces for completion of analysis at a later time after coding to reduce bias. Records of the blood pressure and heart rate measured with oscillometry (Dinamap) were made, and the ventilator settings were not changed. No babies were paralysed, but a detailed analysis of the infant's interaction with the ventilator was not attempted in this study.

Statistical analysis used the Wilcoxon signed rank test and the sign test for paired observations.

Results

The coefficient of variation in cerebral blood flow velocity fell significantly after treatment with dopamine and after plasma: from a median of 13% to 4% after dopamine (p=0·01, Wilcoxon) and from a median of 13% to 5% after plasma (fig 1). Dopamine but not plasma was followed by a significant increase in systolic blood pressure (p=0·01 dopamine, p=0·36 plasma, fig 2). Recordings from one

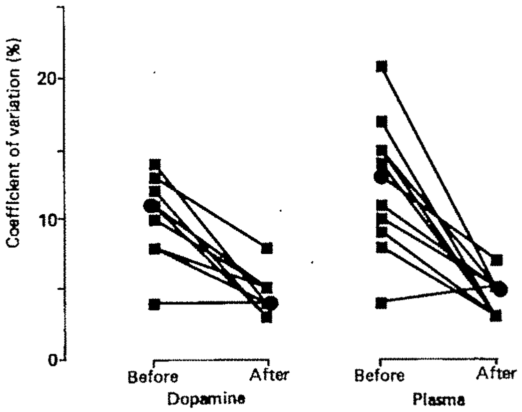

Fig 1 *Coefficient of variation of cerebral blood flow velocity before and after dopamine and plasma. Median values shown as circles.*

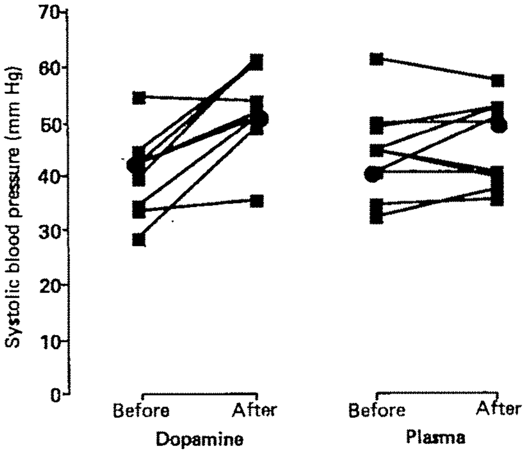

Fig 2 *Sytolic blood pressure (mm Hg) before and after dopamine and plasma. Median values shown as circles.*

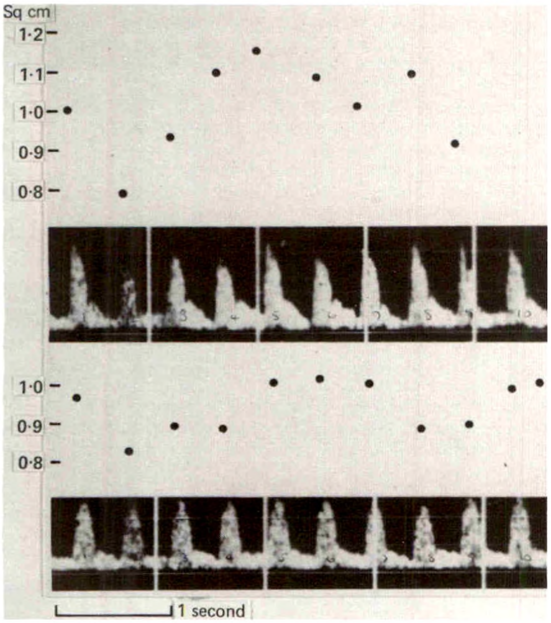

Fig 3 *Spectral analysis of Doppler shift in one baby before (above) and after (below) the commencement of a dopamine infusion.*

Table *Cardiac output and cerebral blood flow velocity before and after dopamine and plasma. Results are medians (interquartile ranges)*

	Cardiac output (ml/kg/minute)	*Cerebral blood flow velocity (cm/second)*
Dopamine:		
Before	149 (133–263)	5·6 (3·2–8·0)
After	154 (126–207)	5·5 (4·0–7·8)
Plasma:		
Before	173 (134–228)	3·8 (2·3–9·4)
After	183 (156–218)	4·2 (3·4–5·4)

baby are shown in fig 3, with the spectral analysis together with the area under the curve for 10 cardiac cycles before and after dopamine. There is a sinusoidal variation corresponding to ventilation which is damped but not abolished by dopamine.

The table shows values for cardiac output and cerebral blood flow velocity. There was no effect on these indices or the heart rate after either form of treatment.

Discussion

These results show that the beat to beat variability of cerebral blood flow velocity was reduced by the administration of plasma and dopamine. Fluctuations seen in the cerebral circulation occurring with positive pressure ventilation are well recognised[18] and reflect the changes in pressure and velocity in the aorta.[4] Fluctuations during spontaneous respiration also occur,[13] and short term alterations in the Pourcelot index of cerebral blood flow velocity at times of fetal breathing greater than those during fetal apnoea have been reported.[19] Fluctuations in systolic blood pressure were more exaggerated in a group of ventilated dogs hypotensive after hypovolaemia than those rendered hypotensive after vasodilator treatment.[20] The effects of plasma in this study were probably due to an increase in circulating blood volume, although it was not possible to measure this directly. These results could provide one explanation for the result of the study of Beverley *et al* using fresh frozen plasma in the protection of periventricular haemorrhage.[21] The provision of coagulation factors by the administration of 10 ml/kg of plasma did not alter in the babies, and were unlikely to have accounted for the beneficial effect observed. If the interpretation of the results presented here is correct and blood pressure fluctuations in the newborn are exaggerated during hypovolaemic hypotension, then the relation between fluctuating cerebral blood flow velocity and periventricular haemorrhage[4 22] occurred probably because low circulating blood volume was a common factor in both periventricular haemorrhage and fluctuating cerebral blood flow rather than fluctuating cerebral blood flow *causing* capillary rupture.

Dopamine could have effectively increased circulating blood volume by increasing systemic vascular resistance and central venous pressure. Babies who received dopamine had invariably already received plasma about an hour previously and were started on an inotrope because of failure to respond, hence the lower systemic pressure seen before treatment in fig 2. The amount of plasma used probably had an insignificant effect on circulating blood volume at this time, however, and an additional explanation for the effect of dopamine has to be sought. The agent is both a direct and indirect beta adrenergic receptor agonist with some alpha adrenergic receptor agonist properties. Several groups have examined the effects in children, in whom an increase or no alteration in central venous pressure and systolic blood pressure occurred.[23] The alpha adrenergic effects predominate at high doses with an increase in systemic vascular resistance.[24] An increase in cardiac output without an increase in heart rate was seen by Padbury *et al*,[25] and although the neonatal myocardium is known to have poor compliance this and the observation in the current study of an increase in systolic blood pressure without an increase in heart rate show that the myocardial response is not limited to rate alone. Work showing an improvement in shortening fraction after dopamine treatment also suggests that the concept that the neonatal heart is at the limit of the Frank-Starling curve may be too simplistic.[26] These results fail to show an increase in cardiac output with the method used despite an increase in systolic blood pressure. Previous studies showing an increase in cardiac output after dopamine have included babies who were shocked before administration with a cardiac output of the order of 100 ml/kg/minute,[27] well below the normal for the newborn.[28] Our threshold for using dopamine is low and the babies reported here entered the study with a cardiac output in the normal range. This or methodological problems could have explained the absence of an increase. The method described has been compared with thermodilution in an animal model, giving correlations of between 0·48 and 0·88, with 95% confidence intervals for the difference in methods ranging from 30 to 40 ml (JM Rennie, RD Barnes. Comparison of cardiac output estimated with Doppler ultrasound and thermodilution in rabbits. Presented at Neonatal Doppler meeting, Leicester, 1987). A Doppler method for cardiac output compared well with a method based on the Fick principle at cardiac catheterisation in children.[17]

The effects of mechanical ventilation on the circulation in sick newborn babies may be more complex than we suspect and remain to be fully explored. The results of this study show a reduction in variability of cerebral blood flow velocity after cardiovascular support and support the observations made in animals that an exaggeration of the variability in systolic blood pressure occurring with ventilation indicates hypovolaemia. Blood pressure is often monitored continuously in premature babies and further analysis of the signal may show a variability which relates to central venous pressure in this way. This could prove of clinical value in neonatal intensive care units where measurement of central venous pressure is difficult.

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References

1 Lou HC, Lassen NA, Friis-Hansen B. Impaired autoregulation of cerebral blood flow in the distressed newborn infant. *J Pediatr* 1979;**94**:118–21.

2 Wimberley PD, Lou HC, Pedersen H, *et al.* Hypertensive peaks in the pathogenesis of intraventricular haemorrhage in the newborn. Abolition by phenobarbitone sedation. *Acta Paediatr Scand* 1982;**71**:537–42.

3 Miall-Allen VM, Vries LS, Whitelaw AGL. Mean arterial blood pressure and neonatal cerebral lesions. *Arch Dis Child* 1987;**62**: 1068–9.

4 Perlman JM, McMenamin JB, Volpe JJ. Fluctuating cerebral blood flow velocity in respiratory distress syndrome. *N Engl J Med* 1983;**309**:204–9.

5 Cooke RWI. Early and late cranial ultrasonographic appearances and outcome in very low birthweight infants. *Arch Dis Child* 1987;**62**:931–7.

6 Guyton AC. *Textbook of medical physiology*. 5th ed. Philadelphia: WB Saunders, 1976:242.

7 Masumi RA, Mason DT, Vera Z, *et al.* Reversed pulsus paradoxus. *N Engl J Med* 1973;**289**:1272–5.

8 Jardin F, Farcot J-C, Gueret P, *et al.* Cyclic changes in arterial pulse during respiratory support. *Circulation* 1983;**68**:266–74.

9 Greenough A, Morley CJ, Davis JA. Interaction of spontaneous respiration with artificial ventilation in preterm babies. *J Pediatr* 1983;**103**:769–73.

10 Cowan F, Thoresen M. The effects of intermittent positive pressure ventilation on cerebral arterial and venous blood velocities in the newborn infant. *Acta Paediatr Scand* 1987;**76**: 239–47.

11 Perlman JM, Goodman S, Kreusser KL, Volpe JJ. Reduction in intraventricular haemorrhage by elimination of fluctuating cerebral blood flow velocity in preterm infants with respiratory distress syndrome. *N Engl J Med* 1985;**312**:1353–7.

12 Rennie JM, South M, Morley CJ. Cerebral blood flow velocity variability in infants receiving assisted ventilation. *Arch Dis Child* 1987;**62**:1247–51.

13 Bignall S, Bailey PC, Rivers RPA, Lissauer TJ. Quantification of cardiovascular instability in premature infants using spectral analysis of waveforms. *Pediatr Res* 1988;**23**:398–401.

14 Perel A, Pizov R, Cotev S. The systolic blood pressure variation is a sensitive indicator of hypovolaemia in ventilated dogs subjected to graded haemorrhage. *Anaesthesiology* 1987;**67**: 498–502.

15 Kitterman JA, Phibbs RH, Tooley WH. Aortic blood pressure in normal newborn infants during the first 12 hours. *Pediatrics* 1969;**44**:959–68.

16 Drayton MR, Skidmore R. Ductus arteriosus blood flow during the first 48 hours. *Arch Dis Child* 1987;**62**:1030–4.

17 Alverson DC, Eldridge M, Dillon T, *et al.* Noninvasive pulsed Doppler determination of cardiac output. *J Pediatr* 1982;**101**: 46–50.

18 Cowan F, Thoresen M. The effects of intermittent positive pressure ventilation on cerebral arterial and venous blood velocities in the newborn infant. *Acta Paediatr Scand* 1987;**76**: 239–47.

19 Wladimiroff JW, van Bel F. Fetal and neonatal cerebral blood flow. *Semin Perinatol* 1987;**11**:335–46.

20 Pizov R, Ya'ari Y, Perel A. Systolic pressure variation is greater during haemorrhage than during sodium nitroprusside-induced hypotension in ventilated dogs. *Anesth Analg* 1988;**67**:170–4.

21 Beverley DW, Pitts-Tucker TJ, Congdon PJ, *et al.* Prevention of IVH by fresh frozen plasma. *Arch Dis Child* 1985;**60**:710–3.

22 Van Bel F, van de Bor M, Stijrien T, *et al.* Aetiological role of cerebral blood flow alterations in development and extension of peri- intraventricular haemorrhage. *Dev Med Child Neurol* 1987;**29**:601–14.

23 Driscoll DJ, Gilette PC, McNamara DG. The use of dopamine in children. *J Pediatr 1978*;**92**:309–14.

24 Driscoll DJ. The use of inotropic and chronotropic agents in neonates. *Clin Pediatr* 1987;**14**:931–48.

25 Padbury JE, Agata Y, Baylen BG, *et al.* Dopamine pharmacokinetics in critically ill newborn infants. *J Pediatr* 1986;**110**: 293–8.

[26] Walther FJ, Siassi B, Ramadan NA, Wu P Y-C. Cardiac output in newborn infants with transient myocardial dysfunction. *J Pediatr* 1985;**107**:781–5.

[27] DiSiessa TG, Leitner M, Ti CC, *et al.* The cardiovascular effects of dopamine in the severely asphyxiated neonate. *J Pediatr* 1981;**99**:772–6.

[28] Walther FJ, Siassi B, Ramadan NA, *et al.* Pulsed Doppler determinations of cardiac output in neonates: normal standards for clinical use. *Pediatrics* 1985;**76**:829–33.

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High alkaline phosphatase activity and growth in preterm neonates

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SUMMARY In a study on 857 infants born preterm, high peak plasma alkaline phosphatase activity was independently related to slower growth rate in the neonatal period, and to a highly significant reduction in attained length at 9 months and 18 months post term. At 18 months the deficit in body length associated with peak neonatal plasma alkaline phosphase activity of 1200 IU/l or more was 1·6 cm (95% confidence interval 0·9 to 2·3 cm) after adjusting for confounding factors. The strength and magnitude of this association between high plasma alkaline phosphase activity and body length was greater than that for any other factor identified, including the infant's sex and the presence of fetal growth retardation. Data are presented that support the view that the high plasma alkaline phosphatase activity reflected early bone mineral substrate deficiency resulting in metabolic bone disease.

We speculate that even silent early bone disease may interfere with the control of subsequent linear growth and emphasise the potential importance of providing preterm infants, especially those fed human milk, with adequate substrate for bone mineralisation.

Metabolic bone disease in preterm infants is common.[1] Though this has often been described as 'rickets', current evidence suggests that in most cases it is caused by a deficiency of mineral substrate rather than of vitamin D.[1–4] It is particularly common in very preterm infants who are being fed on breast milk.[5] In most instances metabolic bone disease is not associated with obvious clinical signs, and the diagnosis has been based on radiographs, photonabsorptiometry, and biochemical changes including high plasma alkaline phosphatase activity. There is little information about whether such asymptomatic bone disease is benign or associated with an adverse outcome. Although there is evidence that linear growth is impaired in classical rickets,[6] the effects of metabolic bone disease on growth performance in premature infants have not been sufficiently investigated.

Preterm infants fed their own mothers' milk have raised plasma alkaline phosphatase activity and reduced linear growth in comparison with formula fed babies,[7] and there is a negative correlation between plasma alkaline phosphatase activity and linear growth at the age of 12 weeks in a small group of preterm infants[8]: there are, however, no longer term studies of growth in relation to early evidence of metabolic bone disease.

In this paper we present such data in 857 infants from a large multicentre outcome study on the long term effects of early diet in low birthweight babies. This cohort has now been studied to 18 months corrected age.

Patients and methods

Infants from five centres (Cambridge, Ipswich, King's Lynn, Norwich, and Sheffield) who were participating in a larger multicentre trial of preterm infant feeding were studied.[9] In all, 857 infants had sequential plasma alkaline phosphatase measurements made, and these are reported here. All infants weighed under 1850 g at birth, and the only exclusions were infants who died within 72 hours of birth and those with serious malformations known to impair growth or development. Parental consent was obtained in all cases, and approval was obtained from the local health authority ethics committees in each of the five centres.

Infants were randomly assigned to diet as described previously.[9] Briefly, infants in three of the centres were assigned to receive banked donor breast milk or a preterm formula (Osterprem) as sole diets (trial 1; n=140) or as supplements to maternal milk (trial 2; n=308). In the other two centres a standard full term adapted formula (Osterfeed) or preterm formula were used as sole diets (trial 3; n=156) or as supplements to maternal milk (trial 4; n=253). The preterm formula contained 35 mg phosphorus and 75 mg calcium/100 ml. The full term formula contained 29 mg phosphorus and 35 mg calcium/100 ml. All infants received a minimum daily vitamin D intake of 800 IU. The median period (quartiles) to attainment of full enteral feeding was 8 (6, 12) days; and to discontinuation of the assigned diet 29 (19, 46) days. After this, infants were either fed by breast or on a standard 'term' infant formula and received 400 IU vitamin D daily until weaning.

The principal objective in this paper was to relate plasma alkaline phosphase activity to neonatal and later growth. Data on the effects of diet on plasma alkaline phosphatase activity and growth are included here only to add weight to the argument that high neonatal alkaline phosphatase activity reflects metabolic bone disease.

Extensive data were collected on obstetric, perinatal, and postnatal events, including the following growth measurements in the neonatal period: body weight (daily to the nearest 1 g on an electronic balance, Sartorius MP series); occipitofrontal head circumference (twice weekly to the next succeeding 1 mm using paper tape); and crown-heel length (once or twice weekly to the next succeeding 1 mm using a horizontal stadiometer). Weight gain (g/kg/day) was calculated from the age when birth weight had been regained. Head growth and length gain were calculated by a similar analysis of measurements made over a period of at least two weeks after regaining birth weight.

Blood samples were drawn weekly at the time of routine monitoring from all infants during their stay in hospital. Plasma calcium concentrations were measured by atomic absorption spectrophotometry and phosphorus was determined by an end point method on 5 μl of plasma using a direct phosphomolybdate reaction without deproteinisation (Smith, Kline Instrument Co Ltd). Alkaline phosphatase activity was measured kinetically on 2·5 μl plasma by the catalysed hydrolysis of p-nitro-phenylphosphate to p-nitrophenol and phosphoric acid (Roche Products Ltd).

All surviving infants from three centres (Cambridge, Ipswich, and King's Lynn) were invited for follow up examinations at 9 months post term, and children from all five centres were examined at 18 months post term. Measurements of weight, crown-heel length, and occipitofrontal head circumference were included at these examinations.

Statistical analysis was by the χ^2 test, Student's *t* test, and regression analysis.

Results

Details of the study population are shown in table 1. The group has been divided into two according to peak plasma alkaline phosphatase activities (≥1200 IU/l). The value of 1200 IU/l, was chosen because, as described below, this was shown to be the cut off point that best discriminated between two groups of infants in terms of their growth performance.

ASSOCIATION BETWEEN INCREASED ALKALINE PHOSPHATASE ACTIVITY AND EVIDENCE OF METABOLIC BONE DISEASE

We used sequential measurement of plasma alkaline phosphatase activity as an enzymatic marker of metabolic bone disease. Previously we conducted a survey in 26 infants (unpublished observations). That showed a highly significant correlation between a score for metabolic bone disease based on radiographic appearances,[10] and maximum plasma alkaline phosphatase activity, confirming the findings of Glass *et al* that alkaline phosphatase activity is a

Table 1 *Clinical details of study population; raw data have been divided according to peak alkaline phosphatase activity (up to, or above 1200 IU/l) during the neonatal period*

	Alkaline phosphatase activity		*p Value*
	<1200 IU/l	*≥1200 IU/l*	
Mean (SEM) birth weight (g)	1420 (11)	1082 (24)	<0·001
Mean (SEM) gestation (weeks)	31·0 (0·1)	29·2 (0·2)	<0·001
Duration of ventilation:			
No (%) <24 hours (n=474)	427 (90)	47 (10)	<0·001
No (%) ≥24 hours (n=381)	298 (78)	83 (22)	
Sex:			
No (%) boys (n=425)	357 (84)	68 (16)	>0·5
No (%) girls (n=432)	370 (86)	62 (14)	
Weight for gestational age:			
No (%) appropriate (n=567)	485 (86)	82 (15)	>0·4
No (%) small for gestational age (n=289)	241 (83)	48 (17)	
Pre-eclampsia:			
No (%) without (n=607)	519 (86)	40 (17)	>0·4
No (%) with (n=240)	200 (83)	40 (17)	

Table 2 *Association between peak plasma alkaline phosphatase activity and incidence of hypophosphataemia, low urinary phosphorus concentration, and hypercalcaemia*

	Peak plasma alkaline phosphatase activity		*p Value**
	<1200 IU/l No (%)	*≥1200 IU/l No (%)*	
Low plasma phosphorus concentration (<1·0 mmol/l)	109/725 (15)	78/130 (60)	<0·0001
†Low urine phosphorus concentration (<0·02 mmol/l)	62/246 (25)	16/36 (44)	<0·02
High plasma calcium concentration (>3·0 mmol/l)	9/549 (1·6)	12/116 (10)	<0·0001

*χ^2 test, ldf; †estimates made on only a segmental subsample.

satisfactory measure of active metabolic bone disease in such studies.[11] We provide here further strong evidence that high plasma alkaline phosphatase activity was related to bone mineral substrate deficiency.

Analysis of raw data showed that infants with high peak plasma alkaline phosphatase activity (≥1200 IU/l) had a higher incidence of low plasma and urinary phosphorus concentrations (minimum recorded values less than 1·0 mmol/l, and 0·02 mmol/l, respectively), and a higher incidence of hypercalcaemia (peak plasma calcium concentration more than 3·0 mmol/l), each suggesting low phosphorus intake or phosphorus deficiency (table 2). These associations were still significant ($p<0·05$) after using logistic regression to adjust for birth weight, gestation, duration of ventilatory assistance, and fetal growth retardation.

In the randomised comparison of infants fed donor breast milk or preterm formula (as sole diets or supplements to maternal milk; trials 1 and 2 combined), those fed banked milk with its lower content of phosphorus and calcium had a higher incidence of high plasma alkaline phosphatase activity (table 3). Fig 1 shows that in infants weighing under 1500 g (the high risk group for bone disease) plasma alkaline phosphatase values remained consistently higher in infants fed donor milk than in those fed a preterm formula throughout the first nine weeks postpartum. High alkaline phosphatase activity in infants fed human milk was associated with a higher incidence of hypophosphataemia and low urinary phosphorus values in this group (table 3).

Table 3 *Effect of donor breast milk or preterm formula, assigned randomly (as sole diets or supplements to maternal expressed milk), on the incidence of high peak plasma alkaline phosphatase values, hypophosphataemia, and low or high urinary phosphorus concentrations*

	Donor breast milk No (%)	*Preterm formula No (%)*	*p Value**
High plasma alkaline phosphatase activity (>1200 IU/l)	57/226 (25)	31/222 (14)	<0·01
Low plasma phosphorus concentration (<1·0 mmol/l)	49/153 (32)	27/155 (17)	<0·01
Low urine phosphorus concentration (<0·02 mmol/l)	39/78 (50)	16/70 (23)	<0·001
High urine phosphorus concentration (>3 mmol/l)	1/78 (1·3)	16/70 (23)	<0·0001

*χ^2, 1 df.

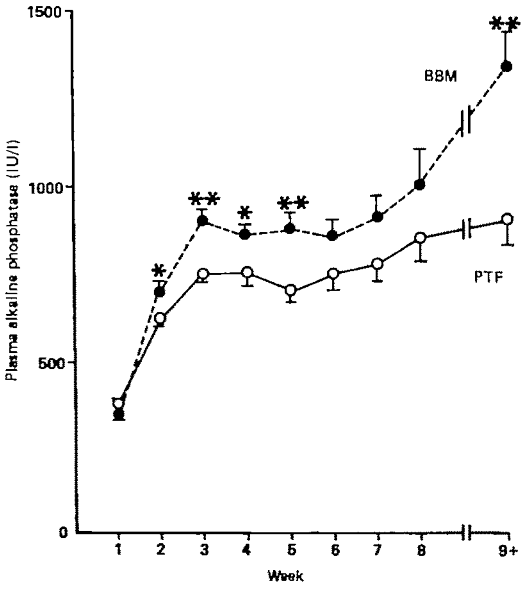

Fig 1 *Mean (SEM) plasma alkaline phosphatase activity from week 1 to week 9 and beyond in infants weighing under 1500 g at birth, according to diet. Infants were randomly assigned to banked breast milk (BBM) or preterm formula (PTF) as sole diets or supplements to their mother's own milk, Sample size at week 1 for BBM was 129 and for PTF 126, falling respectively (as the infants were discharged or died) to 101 and 95 at week 4 and to 36 and 19 at week 9 and beyond; *$p<0·05$; **$p<0·01$.*

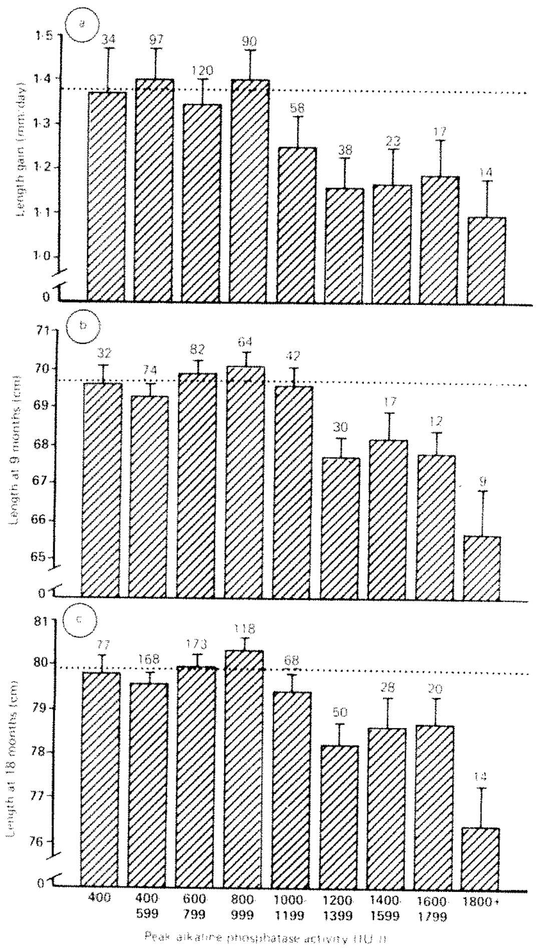

Fig 2 *Association between peak plasma alkaline phosphatase activity and (a) rate of neonatal length gain (b) length at 9 months corrected age and (c) length at 18 months corrected age. The horizontal line represents the mean value for length gain or length at 9 or 18 months in all infants whose peak alkaline phosphatase value was <1000 IU/l (first four data bars of each group).*

ASSOCIATION BETWEEN INCREASED PLASMA ALKALINE PHOSPHATASE ACTIVITY AND GROWTH

Growth was assessed in the neonatal period as weight gain (g/kg/day), length gain (mm/day), and head circumference gain (mm/day). Body weight (kg), length (mm), and head circumference (mm) were recorded at 9 months in three of the five centres, and at 18 months in all centres. The 719 subjects seen at 18 months represented a 94% follow up rate among survivors.

The principle outcome of interest in this study was linear growth. Analysis of raw data (fig 2) indicated that there was no trend in neonatal length gain or in length at 9 at 18 months with increasing peak alkaline phosphatase activities up to 1000 IU/l. When values were above this, linear growth and later body length were lower with the most dramatic reductions seen in babies with peak alkaline phosphatase activites of over 1200 IU/l.

In table 4 raw data are presented for these growth variables according to whether or not peak alkaline phosphatase exceeded 1200 IU/l. In the group of infants with high peak plasma alkaline phosphatase activity, gains in weight, length, and head circumference were significantly reduced in the neonatal period. At 9 and 18 months body length and body weight, though not head circumference, were significantly lower.

These comparisons of raw data are confounded by other differences (including those shown in table 1) between infants with high and low alkaline phosphatase activities, which could contribute to differences in growth performance. We therefore constructed a series of regression models, each of which included one of the above growth variables as the dependent variable; independent variables were plasma alkaline

Table 4 *Association between high plasma alkaline phosphatase activity (peak value >1200 IU/l), growth in the neonatal period, and anthropometric indices at 9 and 18 months (corrected post term age). Data expressed as mean (SEM)*

	Peak plasma alkaline phosphatase activity		*p Value*
	<1200 IU/l	*≥1200 IU/l*	
Neonatal period*:			
Weight gain (g/kg per day) (n=703)	14·6 (0·2)	13·7 (0·2)	<0·02
Head circumference gain (mm/day) (n=581)	1·36 (0·03)	1·16 (0·04)	<0·01
Length gain (mm/day) (n=481)	1·40 (0·02)	1·29 (0·03)	<0·05
At 9 months (n=362):			
Weight (kg)	8·03 (0·07)	7·35 (0·13)	<0·001
Head circumference (mm)	45·5 (0·1)	45·3 (0·2)	>0·4
Length (mm)	69·7 (0·2)	67·6 (0·4)	<0·001
At 18 months (n=719):			
Weight (kg)	10·21 (0·05)	9·68 (0·13)	<0·001
Head circumference (mm)	47·9 (0·1)	47·8 (0·2)	>0·7
Length (mm)	79·8 (0·1)	78·2 (0·4)	<0·001

*Number of subjects analysed for weight, head circumference, and length gain were the number for whom more than three data points had been collected over at least two weeks beyond the age of regaining birth weight.

phosphatase activity (either as a continuous or dichotomous variable), together with birth weight, gestation, fetal growth retardation (above or below 10th centile of birth weight for gestation), sex, days of ventilation, and days in oxygen (expressed as logarithm, plus 1 to avoid log 0), number of fetuses (singletons or twins/triplets) and maternal hypertension. As a further independent variable, diet (exclusively fed human milk or not) was added to the models.

Plasma alkaline phosphatase activity was initially entered into these models as a continuous variable to assess its relationship, after adjusting for potentially confounding factors, with neonatal length gain, and body length, at 9 or 18 months. Though there was a significant linear relationship between increasing peak alkaline phosphatase activity and two of these growth variables (neonatal length gain, $p<0.01$, and length at 18 months, $p<0.0005$), there was a better fit using a quadratic regression, with the square of peak alkaline phosphatase activity relating significantly to length gain ($p<0.01$), length at 9 months ($p<0.01$), and length at 18 months ($p<0.0001$). Nevertheless, from further modelling, and inspection of the raw data in fig 3, we observed a dramatic reduction in neonatal length gain and in body length at 9 or 18 months in infants whose peak alkaline phosphatase was above 1000 IU/l. This justifies treating peak alkaline phosphatase activity as a dichotomous variable. Using a variety of cut off values, significant relationships between peak alkaline phosphatase and all three of the linear growth outcome variables was found for cut off values between 900 IU/l and 1350 IU/l; the best modelled cut off for predicting outcome, however (taking both the size of the regression coefficient and significance into account) was for a cut off at 1200 IU/l (as suggested also by the data in fig 3). In subsequent analysis this value of 1200 IU/l was used to divide infants into those with 'high' or 'low' peak alkaline phosphatase activities.

In the neonatal period (up to the time of discharge) the only factors among those included in the regression model that were independently related to length gain were high alkaline phosphatase activity ($p<0.01$), and diet (<0.05), both of which seemed to have a small additional association with linear growth independent of its relationship with

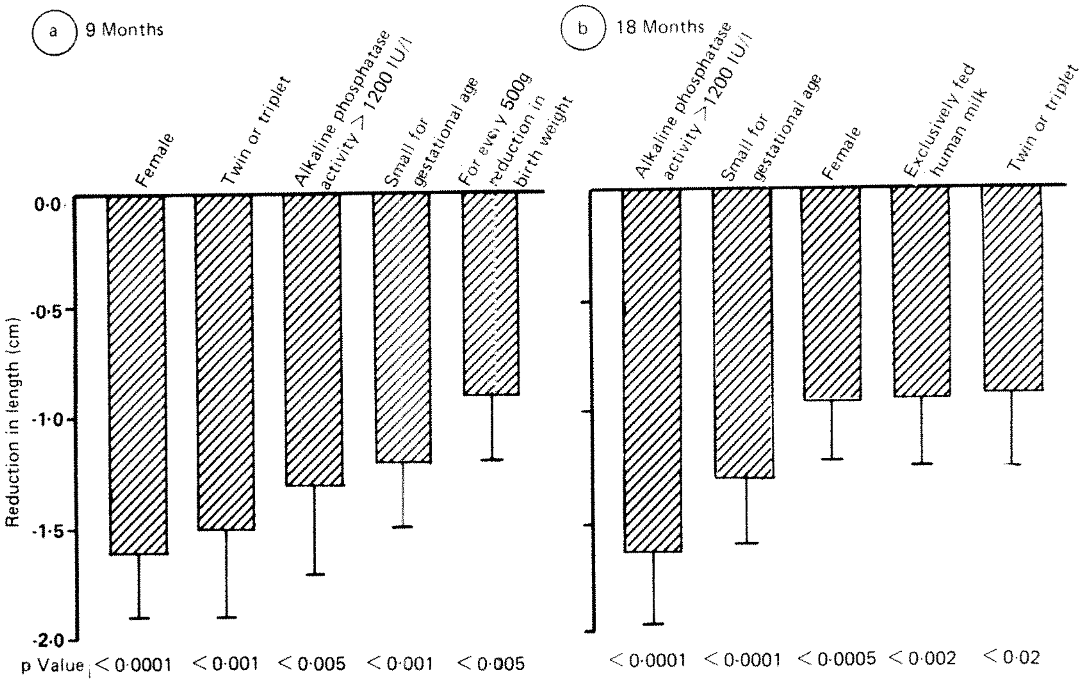

Fig 3 *Factors independently associated with reduced body length at (a) 9 months and (b) 18 months' after term. The magnitude (regression coefficient) of the associations and standard errors depicted were derived from a regression model constucted for each age group, with body length as the dependent variable.*

bone disease. The reduction in length gain in infants with a plasma alkaline phosphatase activity of 1200 IU/l or more was 1·4 mm/week (95% confidence interval (CI) 0·4 to 2·4).

At 9 months the reduction in body length associated with an alkaline phosphatase of 1200 IU or more, after adjusting for the other factors in the model, was 1·3 cm (95% CI 0·4 to 2·2 cm); other factors in the model independently related to reduced body length at 9 months were female sex, low birth weight, and being small for gestational age, or a twin. The magnitude of these associations are shown in fig 3 (a).

At 18 months the association between high neonatal alkaline phosphatase activity and reduced attained length was stronger and of greater magnitude than any other factor identified, which included being female, small for gestational age, or a twin. There was also an association between shorter stature and being fed on human milk in the neonatal period, which seemed to be independent of the association between diet and early bone disease. These associations are shown graphically in fig 3 (b).

The magnitude of the reduction in body length at 18 months associated with a peak neonatal alkaline phosphatase activity of 1200 IU/l or more was 1·6 cm (95% CI 0·9 to 2·3 cm).

When adjustment was made for confounding factors there was no significant association between high alkaline phosphatase activity and gain in weight or head circumference in the newborn period, though there was a strong trend towards a negative association with weight gain, nor was any association with body weight or head circumference found at 9 and 18 months.

Discussion

High neonatal plasma alkaline phosphatase activity in low birthweight infants was strongly associated with impaired linear growth (reduced length gain in the neonatal period) and, of most importance, smaller attained length at 9 and 18 months corrected age. After adjusting for a wide range of potentially confounding factors, high neonatal plasma alkaline phosphatase activity (1200 IU/l or more) emerged as the major factor relating to stature at 18 months. At this age the deficit in length associated with high plasma alkaline phosphatase activity was 1·6 cm, corresponding to more than half a standard deviation for body length in healthy 18 month old children.

Other factors shown to be independently associated with length at 9 or 18 months were sex, being small for gestational age, or a twin (or triplet). There was also an independent association between reduced attained length at 18 months and exclusive human milk feeding, even after adjusting for the effect of this diet on the incidence of metabolic bone disease. Thus for either sex, singleton infants who were an appropriate weight for gestation, fed on a formula, and with a peak plasma alkaline phosphatase activity of less than 1200 IU/l, has a mean length at 18 months close to the 50th centile. In contrast, infants falling into the opposite categories, were estimated to be nearly 4 cm shorter (fig 2), with a mean length near the third centile for an infant born at full term. Maternal pre-eclampsia, which has been reported to cause congenital rickets[12] was not found to be associated with reduced linear growth. This does not rule out, however, an important effect of pre-eclampsia on neonatal mineral homoeostasis and later growth. Such an effect might be entirely subsumed into the effect of being small for gestational age.

A significant association between high alkaline phosphatase activity and reduced short and longer term linear growth was found whether peak alkaline phosphatase was treated as a continuous variable or a dichotomous one. More detailed analysis, however, showed minimal change in linear growth with peak alkaline phosphatase values up to 1000 IU/l, and a precipitous reduction in linear growth if the peak alkaline phosphatase activity was above 1200 IU/l. Thus we suggest that, for prognostic purposes, it is most useful to treat alkaline phosphatase as a dichotomous variable with 'high' and 'low' values above and below 1200 IU/l.

It is important to consider what a raised plasma alkaline phosphatase activity means, as doubt has been thrown on its value as an indicator of metabolic bone disease in low birthweight infants.[13 14] Such doubts have been mainly concerned with its specificity as an indicator of active bone disease at a particular point in an infant's clinical course, and several workers have found values that were not grossly increased at times when there was radiological evidence of frank bone disease.[13 15 16]

The biology of alkaline phosphatase has been reviewed by Anderson.[17] The enzyme is located on the membrane of osteoblast derived 'matrix vesicles', and in parent osteoblasts is close to the mineralisation front. Current evidence suggests that membrane phosphatase is a phosphotransferase, which transfers phosphate residues into matrix vesicles, where—together with trapped calcium ions—they initiate crystallisation. As mineralisation proceeds, both membrane vesicles and osteoblasts are disrupted, and it may be mainly at this stage that alkaline phosphatase leaks into the circulation. Extrapolating from data from Anderson, working on phosphate depleted rats (unpublished observations) we hypothesise that when bone mineral substrate is deficient an adaptive increase in synthesis or activity of alkaline phosphatase occurs, resulting in greatly increased plasma activity despite reduced bone mineralisation. Certainly it would not be surprising if the complex events leading to increased alkaline phosphatase activity in the plasma were not precisely related in time to structural evidence of bone mineral deficiency. Nevertheless, when grossly elevated, plasma alkaline phosphatase activity in neonates is predominantly of bone origin[18] and there are abundant data to show that its activity is usually increased during frank metabolic bone disease, often exceeding the maximum adult value several fold.[11 13-16 19-22] It is increased when bone mineral intake is inadequate and growth is slowed.[2 7 23-26] Many workers have regarded raised plasma alkaline phosphatase activity as an important indicator of metabolic bone disease,[11 16 21 27] and in particular when sequential monitoring is undertaken.[16] Conversely, a reduction in plasma alkaline phosphatase activity has been observed during catch up growth following periods of slow growth in preterm infants.[8]

Our data add further weight to the view that high plasma alkaline phosphatase activity is associated with bone mineral substrate deficiency. In a randomised comparison infants fed human milk had significantly higher plasma alkaline phosphatase activity than those receiving a formula providing twice the calcium and phosphorus contents of breast milk (fig 1). The human milk fed group showed further evidence of phosphorus depletion, with a significantly higher incidence of hypophosphataemia and low urine phosphorus concentrations. Moreover, high alkaline phosphatase activity itself was associated with hypophosphataemia, low urinary phosphorus concentrations, and an increased incidence of hypercalcaemia, which has been described with phosphorus depletion.[28 29]

Regardless of the debate on whether high plasma alkaline phosphatase activity is a valuable neonatal screening test for metabolic bone disease in individual preterm infants, we have shown that it is an important indicator of outcome in terms of later linear growth. Few previous studies have related early bone disease to long term outcome; work in this field has focused mainly on identification and quantification of active bone disease. Metabolic bone disease in preterm infants, however, is usually asymptomatic and is frequently missed. Indeed in this investigation high plasma alkaline phosphatase activity often occurred when the baby was well, and might have been overlooked had the infant not been taking part in a scientific study. We suggest poor linear growth performance both in the short and longer term could be viewed as an important, adverse, clinical outcome response to even silent early metabolic bone disease. We speculate also that the trend towards reduced neonatal weight gain seen with increased plasma alkaline phosphatase activity is linked to the effects of phosphorus depletion on soft tissue growth and metabolism.[30] Though we have shown that high alkaline phosphatase activity relates well to these adverse responses, it is pertinent to note that other tests for metabolic bone disease, namely radiographs of long bones and photon-absorptiometry, as yet have unknown predictive value for later outcome. Furthermore, radiography and densitometry both include irradiation of the patient.

Our hypothesis, strongly supported by the data presented, that neonatal metabolic bone disease has a long lasting and important effect on linear growth, is perhaps a surprising one as excellent catch up is usually seen during the treatment of classical rickets.[31] Early bone disease in preterm infants seems increasingly unlikely to be a vitamin D related problem in most cases[1] so cannot be directly compared with classical rickets. Even so, if we assume that the cause of metabolic bone disease in primarily a deficiency of bone mineral substrate, it still requires explanation that catch up growth and a return to normal growth velocity do not apparently take place once calcium and phosphorus intake and absorption have reached normal infant values[32] and the bones have remineralised.[33]

We have considered that our findings could be explained by some completely different and as yet unidentified confounding factor or factors that were associated with early bone disease and had a continued influence on growth. Arguably it is difficult to adjust statistically for the variable degree of illness seen in preterm infants. We used days of ventilation and days in oxygen as such indicators. Though these measures (especially days of ventilation) have proved to be sensitive markers of poor longer term prognosis for other outcome measures in this cohort (for example neurodevelopment[34]), interestingly we found here that requirement for respiratory support was unrelated to linear growth up to 18 months after adjustment was made for other factors such as birth weight, fetal growth retardation, and twinning. Alternatively, we suggest that early metabolic bone disease itself may interfere with the control mechanisms for subsequent linear growth. This might occur either as a direct consequence of the bone disease itself, or because of the secondary effects such disease may have on the hormonal milieu during a critical growth period normally protected in the intrauterine environment. Indeed, it is possible that any process that results in lasting retardation of linear growth at an early—perhaps critical—stage could have an adverse 'programming' or 'imprinting' effect on later linear growth performance. In this regard it is worth considering the serious effect on long term linear growth that may occur in infants who have been born stunted at term after prolonged placental insufficiency.[35] Our data presented here indicate an independent detrimental effect of fetal growth retardation on linear growth to 18 months.

We suggest that even entirely subclinical metabolic bone disease may have prolonged consequences for growth performance. Though our future planned follow up of this cohort will be important in assessing the longer term significance of our observations, at present it would seem prudent to make every effort to achieve an optimal intake of bone mineral substrate in the neonatal period. This cannot be achieved with unsupplemented human milk.

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References

[1] Brooke OG, Lucas A. Metabolic bone disease in preterm infants. *Arch Dis Child* 1985;**60:**682–5.

[2] Rowe J, Rowe D, Horak E, *et al.* Hypophosphatemia and hypercalciuria in small premature infants fed human milk: evidence for inadequate dietary phosphorus. *J Pediatr* 1984;**104:** 112–7.

[3] Chan GM, Mileur L, Hansen JW. Effects of increased calcium and phosphorus formulas and human milk on bone mineralization in preterm infants. *J Pediatr Gastroenterol Nutr* 1986;**5:**444–9.

[4] Gross SJ. Bone mineralization in preterm infants fed human milk with and without mineral supplementation. *J Pediatr* 1987;**111:**450–8.

[5] McIntosh N, Livesay A, Brooke OG. Plasma 25-hydroxyvitamin D and rickets in infants of extremely low birthweight. *Arch Dis Child* 1982;**57:**848–50.

[6] Stearns G, Jeans PC, Vandecar V. The effect of vitamin D on linear growth in infancy. *J Pediatr* 1936;**9:**1–10.

[7] Cooper PA, Rothberg AD, Davies VA, Argent AC. Comparative growth and biochemical response of very low birthweight infants fed own mother's milk, a premature infant formula, and one of two standard formulas. *J Pediatr Gastroenterol Nutr* 1985;**4:**786–94.

[8] James JA, Mayne PD, Barnes IC, Kovar IZ. Growth velocity and plasma alkaline phosphatase activity in the preterm infant. *Early Hum Dev* 1985;**11:**27–32.

[9] Lucas A, Gore SM, Cole TJ, *et al.* Multicentre trial on feeding low birthweight infants: effects of diet on early growth. *Arch Dis Child* 1984;**59:**722–30.

[10] Pohlandt F. A radiological sign of bone demineralization in preterm infants. *Klin Padiatr* 1985;**197:**155–6.

[11] Glass EJ, Hume R, Hendry GM, Strange RC, Forfar JO. Plasma alkaline phosphatase activity in rickets of prematurity. *Arch Dis Child* 1982;**57:**373–6.

[12] Zeidan S, Bamford M. Congenital rickets with maternal pre-eclampsia. *J R Soc Med* 1984;**77:**426–7.

[13] McIntosh N, Williams JE, Lyon AJ, Wheeler KA. Diagnosis of rickets of prematurity. *Lancet* 1984;**ii:**869.

[14] James JR, Congdon PJ, Truscott J, Horsman A, Arthur R. Osteopenia of prematurity. *Arch Dis Child* 1986;**61:**871–6.

[15] Koo WW, Gupta JM, Nayaner VV, Wilkinson M, Posen A. Skeletal changes in preterm infants. *Arch Dis Child* 1982;**57:** 447–52.

[16] Walters EG, Murphy JF, Henry P, Gray OP, Elder GH. Plasma alkaline phosphatase activity and its relation to rickets in preterm infants. *Ann Clin Biochem* 1986;**23:**652–6.

[17] Anderson HC. Matrix vesicle calcification: review and update. In: Peck WA, ed. *Bone and mineral research 3.* Amsterdam: Elsevier, 1985:109–49.

[18] Brooke OG, Brown IRF, Bone CDM, *et al.* Vitamin D supplements in pregnant Asian women: effects on calcium status and fetal growth. *Br Med J* 1980;**280:**751–4.

[19] Kovar IZ, Mayne P, Wallis J. Neonatal rickets in one of identical twins. *Arch Dis Child* 1982;**57:**792–4.

[20] Chudler AE, Brown DR, Holzman IR, Oh KS. Nutritional rickets in two very low birthweight infants with chronic lung disease. *Arch Dis Child* 1980;**55:**687–90.

[21] Kovar IZ, Mayne P, Barltrop D. Plasma alkaline phosphatase activity: a screening test for rickets in preterm neonates. *Lancet* 1982;**i:**373–6.

[22] Callenback JC, Sheehan MB, Abramson SJ, Hall RT. Etiologic factors in rickets of very low-birthweight infants. *J Pediatr* 1981;**98:**800–5.

[23] Eek S, Gabrielsen LH, Halvorsen S. Prematurity and rickets. *Pediatrics* 1957;**20:**63–77.

[24] Schanler RJ, Garza C, O'Brien Smith E. Fortified mother's milk for very low birthweight infants: results of macromineral balance studies. *J Pediatr* 1985;**107:**767–74.

[25] Hillman LS, Hoff NH, Salmons S, Martin L, McAlister W, Haddad J. Mineral homeostasis in very premature infants: serial evaluation of serum 25-hydrocyvitamin D, serum minerals, and bone mineralization. *J Pediatr* 1985;**106:**970–80.

[26] Pettifor JM, Stein H, Herman A, Ross FP, Blumenfield T, Moodely GP. Mineral homeostasis in very low birthweight infants fed either own mother's milk or pooled pasteurized preterm milk. *J Pediatr Gastroenterol Nutr* 1986;**5:**248–53.

[27] Hillman LS, Salmons SJ, Slatopolsky E, McAlister WH. Serial serum 25-hydroxyvitamin D and mineral homeostasis in very premature infants fed preterm human milk. *J Pediatr Gastroenterol Nutr* 1985;**4:**762–70.

[28] Miller RR, Menke JA, Mentser MI. Hypercalcemia associated with phosphate depletion in the neonate. *J Pediatr* 1984;**105:** 814–7.

[29] Lyon AJ, McIntosh N, Wheeler K, Brooke OG. Hypercalcaemia in extremely low birthweight infants. *Arch Dis Child* 1984;**59:** 1141–4.

[30] Wharton BA. *Nutrition and feeding of preterm infants.* Oxford: Blackwell, 1987:117–132.

[31] Hess AF. *Rickets including osteomalacia and tetany.* London: Henry Kimpton, 1930: 401–29.

[32] Salle B, Senterre J, Putet G, Rigo J. Effects of calcium and phosphorus supplementation on calcium retention and fat absorption in preterm infants fed pooled human milk. *J Pediatr Gastroenterol Nutr* 1986;**5:**638–42.

[33] Chan GM, Mileu LJ. Post hospitalization growth and bone mineral status of normal preterm infants. *Am J Dis Child* 1985;**139:**896–8.

[34] Lucas A, Morley R, Cole TJ. Adverse neurodevelopmental outcome of moderate neonatal hypoglycaemia. *Br Med J* 1988;**297:**1304–8.

[35] Neligan GA, Kolvin I, Scott DM, Garside RF. Born too soon or born too small. *Clinics in developmental medicine* No 61. London: Heineman, 1976:54–60.

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Bone mineral accretion rate and calcium intake in preterm infants

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SUMMARY Thirty six preterm infants (20 boys) of 25 to 32 weeks' gestation were observed from birth to around 40 weeks' postconception. When oral feeding became possible, nine received mother's own breast milk (group B), 15 formula feed (group F), and 12 formula feed supplemented with calcium (5 ml 10% calcium gluconate/100 ml feed) and phosphorus (0·5 ml 17% potassium phosphate similarly) (group S). All received a daily supplement of 400 IU vitamin D. Intakes of calcium, phosphorus, vitamin D, energy, and fluid volume were recorded. When oral feeding started, and near 40 weeks' postconception, bone mineral content of the forearm was measured by photon absorptiometry; weight and crown-heel length were also measured.

After logarithmic transformation of the measurements, there were no significant intergroup differences between the mean rate constants for weight or crown-heel length describing growth during the observation period. The mean rate constant for mineral accretion (M) was significantly higher in group S than in both the others. Pooling all data, M was significantly correlated with calcium intake but not with any other variable. Mineral supplementation of feed can reduce but not cure osteopenia of prematurity.

By applying single photon absorptiometry to the mid forearm of neonates,[1] we have previously shown the existence of a substantial deficit in bone mineral content, relative to full term infants observed at birth, in most preterm infants when they reach a postconceptional age of 40 weeks.[2 3]

Bone mineral content of cortical bone is dependent on both density of mineralisation and bone size in the measurement region. Infants with extremely low birth weight (ELBW) (that is, birth weight less than 1000 g) have recently been shown to be at a disadvantage in both respects.[3] At about 40 weeks they are underweight by 45%, shorter by 20%, and undermineralised by 55% relative to full term controls observed at birth; taking into account their reduced body size, at 40 weeks ELBW infants are still undermineralised by 35%. Preterm infants with birth weights in excess of 1000 g have also been shown to be undermineralised at 40 weeks by about 20% when body size reduction is taken into account.[2] Numerical values of relative undermineralisation observed in such studies, based as they were on localised measurements of the peripheral skeleton, do not necessarily apply to the skeleton as a whole; none the less, a mineral deficit in the forearm bones of a preterm infant is usually indicative of more widespread osteopenia.

Radiographically detectable osteopenia of prematurity has reportedly been ameliorated by vitamin D supplementation.[4] However, radiological assessment detects only the most severe cases. In contrast, our measurements of the effects of vitamin D supplementation of randomly selected preterm infants did not show a significant improvement in the rate of postnatal mineral accretion when the vitamin D intake was raised from the standard 400 IU/day to 1000 IU/day (J James, PJ Congdon, A Horsman, *et al.* Unpublished observations). In the same study, infants given 1α-hydroxyvitamin D had a slightly raised rate of mineral accretion but still did not reach values comparable with those attained in utero.[5]

There is, however, some evidence that bone mineral accretion can be accelerated by mineral supplementation,[6] and given the lack of response to vitamin D supplements we consequently gave calcium and phosphorus supplemented feeds to some of the preterm infants in our neonatal unit. In this paper we present observations of changes in bone mineral content in preterm infants some of whom were given breast milk, the remainder being offered either normal feeds or feeds supplemented with calcium and phosphorus, the aim being to determine whether mother's milk or increased mineral intake conferred detectable benefits with respect to mineral accretion rate and general growth parameters.

Patients and methods

This study was authorised by the local ethical committee and commenced in September 1986, being carried out over a period of 17 months. During that period, all white infants of less than 33 weeks' gestation who were born in The General Infirmary, Leeds, or transferred to our neonatal unit during the first week of life, were considered eligible for the study. Those who were transferred back to their referring hospitals before the initial assessment could be performed were excluded.

Parents of 36 infants consented to the recruitment of their child into the study, having been approached soon after the birth. Gestational age (completed weeks) and birth weight were noted in each case. Gestational age, calculated from maternal menstrual history and confirmed by either external examination[7] or ophthalmoscopic examination of the lens,[8] ranged from 25 to 32 weeks and birth weight from 650 to 2000 g. Descriptive statistics for observations at birth are contained in table 1 for each of the three dietary groups described below.

Most infants received intravenous fluids immediately after birth and, if fluids were required for three days or more, total parenteral nutrition was then implemented using standard solutions supplying up to 60 mg/kg/day calcium, 40 mg/kg/day phosphorus, and 200 IU/day vitamin D. When the infants were able to tolerate oral feeds they were allocated to one of three dietary groups: breast milk, formula fed, and supplemented groups denoted by B, F, and S, respectively.

Mothers who wished to feed their infants with breast milk were allowed to do so. The infants in this group, B, received expressed milk until able to feed at the breast. Nine infants (seven boys) out of the 36 participants in this study were fed in that way throughout the individual period of observation. Each received an oral daily supplement of 400 IU vitamin D.

Those infants whose mothers did not wish to breast feed were given standard cows' milk formula feeds, including the standard daily supplement of 400 IU vitamin D, with or without additional mineral supplementation. The unsupplemented feed contained calcium and phosphorus in concentrations in the ranges 44 to 61 and 27 to 50 mg/100 ml respectively and was received by 15 infants (six boys) comprising group F. The additional supplement was allocated on a random basis and consisted of 5 ml 10% calcium gluconate plus 0·5 ml 17·4% dipotassium hydrogen phosphate added to each 100 ml bottle of ready made feed. Concentrations of calcium and phosphorus in the supplemented feed, which was received by 12 infants (seven boys) comprising group S, were in the ranges 86 to 102 and 46 to 62 mg/100 ml of prepared feed respectively; dilution caused a reduction in energy content of about 5%.

Infants in group S were screened for hypercalcaemia or hyperphosphataemia on a weekly basis. No deleterious effects of this form of supplementation were observed. When the supplemented infants were discharged from the neonatal unit, mineral supplements were discontinued in view of the potential errors and dangers involved in adding mineral supplements at home. The infants then received standard cows' milk formula feed.

For each infant, calcium, phosphorus, vitamin D, energy, and fluid intakes were assessed for every day in the period between the two absorptiometric scans (see below). Calcium and phosphorus concentrations in breast milk were taken to be 25 and 17 mg/100 ml respectively, based on analyses of pooled samples from eight mothers. Most of the informa-

Table 1 *Descriptive statistics for gestational age and weight at birth in the breast milk group (B), the formula fed group (F), and the group of infants given calcium and phosphorus supplemented feeds (S)*

	Group B (n=9)		*Group F (n=15)*		*Group S (n=12)*	
	Gestational age (weeks)	*Birth weight (g)*	*Gestational age (weeks)*	*Birth weight (g)*	*Gestational age (weeks)*	*Birth weight (g)*
Mean	28·22	1219	29·67	1254	30·17	1426
SD	1·64	288	2·16	316	1·85	296
Minimum	25	925	26	950	27	650
Maximum	30	1750	32	2000	32	1800

tion about intakes was obtained from dietary records; however, for each infant discharged from the neonatal unit before the second scan, the dietary intakes after discharge were estimated from a diet history taken when the second scan was performed. Mean values of calcium and phosphorus intakes in mg/kg/day, vitamin D intake in IU/day, energy intake in MJ/kg/day (except in group B) and fluid intake in ml/kg/day were evaluated for each infant. Individual total calcium and phosphorus intakes were divided by individual observation periods to provide average rates of nutrient intake in g/week. Descriptive statistics for the dietary variables are given in table 2.

In all infants within seven weeks of birth, and in most (29 out of 36) within three weeks of birth, bone mineral content was measured at the middle of the right forearm by photon absorptiometry as described in previous publications.[1] [2] The radiation exposure involved in this procedure is very small, and only a narrow area of the forearm is irradiated during a scan; skin entrance dose is 0·03 mGy per

Table 2 *Descriptive statistics for estimates of dietary intakes of calcium, phosphorus, vitamin D, energy, and fluids for the three dietary groups between the first and second absorptiometric scans*

	Calcium			*Phosphorus*			*Vitamin D*		*Energy (MJ/kg/day)*	*Fluids (ml/kg/day)*
	mg/kg/day	*g*	*g/week*	*mg/kg/day*	*g*	*g/week*	*IU/day*	*IU*		
Group B (n=9):										
Mean	52·31	7·35	0·713	36·87	5·14	0·501	400	27820	—	187·6
SD	7·62	3·47	0·264	5·91	2·42	0·192	—	5656	—	20·9
Minimum	39·40	2·38	0·415	29·10	1·75	0·303	400	15988	—	149·4
Maximum	67·30	12·98	1·218	49·90	8·80	0·907	400	35196	—	216·7
Group F (n=15):										
Mean	92·49	7·52	1·035	63·51	5·29	0·721	483·9	25467	0·483	177·6
SD	20·32	3·25	0·361	15·71	2·73	0·313	79·7	7630	0·041	15·7
Minimum	51·30	2·72	0·464	41·80	1·59	0·232	311·0	12757	0·400	146·6
Maximum	113·30	12·40	1·621	92·20	10·17	1·329	595·0	42115	0·556	202·0
Group S (n=12):										
Mean	136·60	12·57	1·690	81·99	7·44	0·994	518·1	26984	0·482	184·3
SD	36·83	5·10	0·374	15·56	3·38	0·267	70·7	9717	0·038	11·0
Minimum	95·60	6·81	1·147	64·70	3·95	0·675	359·0	14644	0·396	167·7
Maximum	233·30	19·86	2·255	112·70	15·45	1·774	618·0	40916	0·544	204·8

Table 3 *Descriptive statistics for observations at the time of the first scan. Weight, crown-heel length, and bone mineral content were measured in the mid forearm at that time for the three dietary groups. Natural logarithms are denoted by 1n*

	Postconceptional age (weeks)	*Weight*		*Crown-heel length*		*Bone mineral content*	
		g	*ln*	*cm*	*ln*	*mg/cm*	*ln*
Group B (n=9):							
Mean	30·29	1188	7·062	38·87	3·658	95·56	4·540
SD	0·72	247	0·203	2·60	0·066	19·90	0·213
Minimum	29·00	910	6·813	35·20	3·561	70·81	4·260
Maximum	31·29	1600	7·378	43·30	3·768	126·41	4·840
Group F (n=15)*:							
Mean	31·92	1252	7·105	39·28	3·669	101·06	4·599
SD	1·45	317	0·236	2·35	0·060	19·26	0·192
Minimum	29·29	825	6·715	35·30	3·564	71·62	4·271
Maximum	35·00	2000	7·601	43·20	3·766	140·93	4·948
Group S (n=12):							
Mean	31·76	1342	7·190	39·92	3·685	101·03	4·604
SD	1·64	214	0·161	2·12	0·055	14·73	0·160
Minimum	28·29	950	6·856	34·60	3·544	66·55	4·198
Maximum	34·00	1700	7·438	43·10	3·764	121·98	4·804

*n=14 for crown-heel length.

investigation. Postconceptional ages at the time of the first scan ranged from 28 to 35 weeks. Weight (in g) and crown-heel length (in cm) were measured at the same time. A crown-heel length measurement was not taken in one case. Descriptive statistics for observations performed at this time are contained in table 3.

Between the postconceptional ages of 37 and 43 weeks bone mineral content, weight, and crown-heel length were measured again. Crown-heel length was not measured in one case; this was a different infant from the one in whom crown-heel length was not measured on the first scan. The interval between the first and second scans ranged from 3·7 to 12·6 weeks (mean 8·0 weeks). Descriptive statistics for observations performed at the time of the second scan are contained in table 4.

STATISTICAL ANALYSIS

Individual values of weight, crown-heel length, and bone mineral content were converted to logarithms to the base *e* because previously published intrauterine data indicated that logarithmic transformation is appropriate[5]; individual infants were assumed to follow exponential growth curves for all three variables during the postconceptional age range covered by these investigations. Individual rate constants (that is, rates of change of the natural logarithms of the measurements) were calculated by dividing the changes in the logarithms by the corresponding time intervals of observation (in weeks) between the first and second scans. Student's *t* test was used to evaluate the significances of observed mean changes within and mean differences between groups; significances of differences in mean rate constants between groups were evaluated similarly. Paired *t* tests were used in comparisons of the preterm infants with full term infants observed previously and matched for postconceptional age at the time of photon absorptiometry.

Results

With one exception, there were no significant intergroup differences in the mean values of gestational age and weight at birth (table 1). Infants in group B had a significantly lower mean gestational age (28·22 weeks) than those in group S (30·17 weeks; $p<0{\cdot}05$).

The data in table 2 show the disadvantage of breast feeding and the effectiveness of supplementation with regard to mineral intakes. As percentages of the mean mg/kg/day value in the group F, calcium and phosphorus intakes were 57% and 58% respectively in group B and 148% and 129% respectively in group S. Intergroup differences in the mean values of intake expressed as mg/kg/day were all highly significant ($p<0{\cdot}01$); with the exception of the difference in phosphorus intake between groups B and F, differences between intakes expressed as

Table 4 *Descriptive statistics for observations at the time of the second scan. Weight, crown-heel length, and bone mineral content were measured in the mid forearm at that time for the three dietary groups. Natural logarithms are denoted by ln*

	Postconceptional age (weeks)	*Weight*		*Crown-heel length*		*Bone mineral content*	
		g	*ln*	*cm*	*ln*	*mg/cm*	*ln*
Group B (n=9):							
Mean	40·22	2685	7·850	46·99	3·845	96·12	4·528
SD	1·67	811	0·332	4·53	0·097	29·60	0·284
Minimum	37·00	1350	7·208	39·70	3·681	66·38	4·195
Maximum	42·86	3760	8·232	53·30	3·976	158·35	5·065
Group F (n=15)*:							
Mean	39·18	2399	7·753	46·20	3·830	98·53	4·575
SD	1·11	580	0·256	3·59	0·079	16·88	0·185
Minimum	37·14	1400	7·244	39·10	3·666	60·88	4·109
Maximum	40·86	3400	8·132	51·80	3·947	126·80	4·843
Group S (n=12):							
Mean	39·13	2453	7·782	46·43	3·836	116·68	4·742
SD	1·66	562	0·222	2·72	0·059	20·26	0·203
Minimum	37·29	1850	7·523	41·00	3·714	64·95	4·174
Maximum	42·71	3600	8·189	51·80	3·947	148·49	5·001

*n=14 for crown-heel length.

g/week were also significant ($p<0.05$). Mean calcium:phosphorus ratio (based on the mg/kg/day figures) was higher in group S (1·67) than in the other two groups (1·42 in group B and 1·46 in group F).

Values given in table 2 for vitamin D intake do not include the component present in the mother's milk fed to group B. The mean intake (IU/day) in group S was higher by about 7% than the mean in group F; this difference is mainly an indirect result of the fact that infants in group S tended initially to be slightly heavier and therefore were offered more feed than those in group F (see the mean weight values in tables 3 and 4). Mean energy intake (MJ/kg/day), however, did not differ between groups F and S (table 2; data were not available for group B), nor were there significant differences between the three groups with respect to fluid intake.

It was anticipated from the way the study was performed that there would be no systematic differences between the groups at the time of the first scan. Results at this time are given in table 3. Although the postconceptional age was significantly lower in group B than in group F ($p<0.01$) and group S ($p<0.05$), the differences between the means were small (less than 1·7 weeks). There were no significant intergroup differences between the mean values of weight, crown-heel length, and bone mineral content, or between the mean values of the logarithms of those measurements at the time of the first scan.

Results at the time of the second scan are given in table 4. Mean values of postconceptional age at that time did not differ significantly between groups and occupied the range 39·13 to 40·22 weeks. Group B was the heaviest (mean 2685 g) but there were no significant weight (or ln weight) differences between groups. Group B was also the longest (mean crown-heel length 46·99 cm) but again there were no significant intergroup differences. Similar mean values of bone mineral content were attained at this time by groups B and F, although the variance was greater in the former. Both mean bone mineral content and its logarithmic equivalent, however, were significantly higher in group S than in group F ($p<0.02$ and $p<0.05$ for bone mineral content and its natural logarithm respectively). The figure summarises the results obtained at the times of the first and second scans.

Mean values of the time interval between birth and the first scan were 2·1, 2·3, and 1·6 weeks for groups B, F, and S, respectively and were not significantly different from one another. For the three groups together, the mean was 2·0 weeks with individual time intervals ranging from 1 day to 7·0 weeks. The interval was less than three weeks in 78% of cases and less than two weeks in 58%. During that period, some infants gained while others lost weight. The rate of weight change for all infants averaged (SEM) −25 (17) g/week and was not significantly different from zero. Individual changes in weight ranged from −280g to +300g. Such changes are consistent with common clinical experience of preterm infants.

Absolute changes in the mean values of the measurements can be derived from tables 3 and 4. The mean (SD) interval between the first and

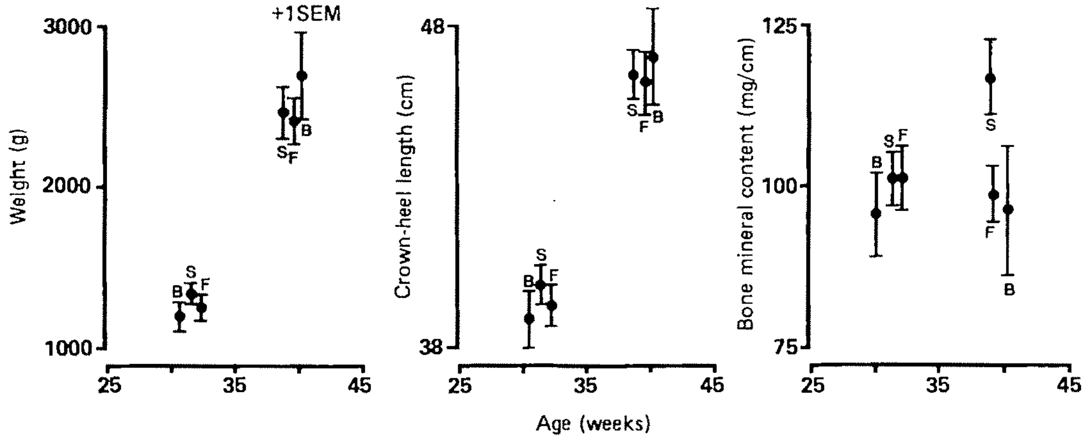

Figure *Mean (SEM) of weight, crown-heel length, and bone mineral content of the mid forearm in the three dietary groups at the times of the first and second absorptiometric scans. B=breast milk, F=formula, and S=supplement.*

second scans varied between groups however, it was 9·94 (2·02) weeks in group B, 7·26 (1·87) weeks in group F, and 7·37 (2·20) weeks in group S. Significant differences between the mean interval in group B and both the other groups (p<0·02) make interpretation of absolute changes difficult. Instead, rates of change of the measurements were compared.

Results derived from individual rates of change of the measurements and individual rate constants are given in tables 5 and 6 respectively. Values in table 5 are the weekly changes in the raw measurements and were evaluated only as a guide to the growth rates. Statistical tests were not performed on those results as it is known that the rate of change of each of the three variables is itself an increasing function

Table 5 *Rates of change (weekly changes in measurements) for weight, crown-heel length, and bone mineral content of the mid forearm in the three dietary groups. Results apply to the period between the first and second scans*

	Rates of change		
	Weight (g/week)	*Crown-heel length (cm/week)*	*Bone mineral content (mg/cm/week)*
Group B (n=9):			
Mean	144·6	0·790	−0·027
SD	45·9	0·251	2·027
Minimum	61·3	0·385	−3·126
Maximum	222·5	1·055	+2·541
Group F (n=15)*:			
Mean	153·9	0·998	−0·560
SD	47·0	0·409	1·782
Minimum	75·0	0·550	−3·234
Maximum	238·1	2·289	+2·510
Group S (n=12):			
Mean	148·5	0·905	2·349
SD	37·6	0·206	2·013
Minimum	80·7	0·631	−1·118
Maximum	211·1	1·356	+5·235

*n=13 for crown-heel length.

Table 6 *Rate constants (weekly changes in natural logarithms of measurements) for weight, crown-heel length, and bone mineral content of the mid forearm in the three dietary groups. Results apply to the period between the first and second scans and were used for evaluation of the significance of intergroup differences of the means*

	Rate constants		
	Weight	*Crown-heel length*	*Bone mineral content*
Group B (n=9):			
Mean	0·0781	0·01836	−0·0018
SD	0·0132	0·00536	0·0207
SEM	0·0044	0·00179	0·0069
Group F (n=15)*:			
Mean	0·0881	0·02319	−0·0057
SD	0·0229	0·00848	0·0202
SEM	0·0059	0·00235	0·0052
Group S (n=12):			
Mean	0·0811	0·02112	0·0207
SD	0·0177	0·00523	0·0175
SEM	0·0051	0·00151	0·0051

*n=13 for crown-heel length.

of postconceptional age; the growth curves are exponential. Mean rate of weight gain was about the same in all three groups (about 150 g/week); within all three groups there was a wide spread in the individual rate of weight gain, with maximum values approximately three times the minimum values. Group B appeared to grow in length least rapidly (see below), mean values of the rate of increase of crown-heel length being similar in the other two groups (about 1 cm/week); again there was wide variability between individuals, with maximum values of the rate of crown-heel length increase being two to four times the minimum values. Based on the rate constants in table 6 there were no significant intergroup differences in weight increase or linear growth. For bone mineral content, however, the mean rate constant was significantly higher in group S than in both the other two groups ($p<0\cdot01$ for groups S and F, and $p<0\cdot02$ for groups S and B). There was no evidence within any group that the distribution of any rate constant was other than normal.

Correlations between rate constants for weight, crown-heel length and bone mineral content were evaluated. Within the groups, there were only two significant correlations, both involving weight and crown-heel length: in group B, $r=0\cdot80$ ($p<0\cdot02$) and in group F, $r=0\cdot62$ ($p<0\cdot05$). Pooling results in the three groups, again only the correlation coefficient between the rate constants for crown-heel length and weight was significant ($r=0\cdot60$; $p<0\cdot0002$). The rate constant for bone mineral content was not significantly correlated with the rate constant for either crown-heel length or weight.

For calcium and phosphorus, individual intakes expressed both as mg/kg/day and g/week (averaged over the period between the two scans) were available. In the case of calcium, pooling the results in the three groups, the rate constant for bone mineral content was significantly correlated with the intake expressed as mg/kg/day ($r=0\cdot43$; $p<0\cdot01$) and as g/week ($r=0\cdot36$; $p<0\cdot05$). In the case of phosphorus, the corresponding associations were not significant ($r=0\cdot22$ and $r=0\cdot09$ respectively).

None of the rate constants for weight, crown-heel length, or bone mineral content (again pooling the results in the three groups) was significantly correlated with energy intake (MJ/kg/day) or fluid intake (ml/kg/day). The correlation coefficient between the rate constant for bone mineral content and vitamin D intake (IU/day) was $0\cdot30$ ($0\cdot05<p<0\cdot1$); the vitamin D intake was not correlated with any other variable at the significance level $p<0\cdot1$. All possible correlations within each group were also evaluated. There were no associations significant at the level $p<0\cdot05$.

It was possible to match, on the basis of postconceptional age at the time of observation, nine of the infants in group S with nine full term white infants observed in previous studies.[2] The matching tolerance was less than one week; seven of the nine pairs were also sex matched. The matching exercise was limited to group S because the expected difference between preterm and full term infants in the mean value of bone mineral content was least in that group. Paired *t* tests were used to examine the significance of differences in the group mean values of weight, crown-heel length, and bone mineral content between the preterm and full term infants. Mean postconceptional age was almost identical in the two groups (39·7 weeks). The preterm infants were significantly lighter (preterm mean 2579 g; full term mean 3470 g; $p<0\cdot01$) and shorter (preterm crown-heel length mean 46·8 cm; full term mean 51·0 cm; $p<0\cdot01$). Despite the fact that supplementation promotes bone mineral accretion, the mean value of bone mineral content in the preterm infants was significantly less than in the matched full term controls (preterm bone mineral content mean 113·9 mg/cm; full term value 211·0 mg/cm; $p<0\cdot001$).

Discussion

Osteopenia of prematurity results from postnatal failure to match the rate of skeletal mineral accretion achieved in utero.[5 6] It is likely that reduced accretion is at least partly a consequence of relative lack of mineral (calcium and phosphorus) in the diet. Supportive evidence for this view is provided by the observation that the mineral supply is particularly deficient in human milk (see table 2), a feed which has previously been shown to be associated with poor calcium retention and an increased risk of osteopenia.[9–11] Furthermore, despite the insensitivity of radiological assessment of skeletal mineralisation,[12] studies based on radiographic examination have shown a very high prevalence of rickets in extremely low birthweight infants fed human milk.[13] Higher activities of alkaline phosphatase in blood, indicative of metabolic bone disease, have also been recorded in premature infants fed human milk.[14]

A reasonable approach to the treatment or prevention of osteopenia, therefore, is to increase dietary mineral intake. When offering a supplemented diet to a preterm infant, however, it has to be accepted that the skeletal response is likely to depend on both the size of the supplement and the formulation of the feed, with variations in mineral bioavailability causing variations in mineral absorption between different formulations.[15] Whatever the response is, it can be measured, albeit with considerable technical difficulty, by calcium balance studies because a large proportion of total body calcium is located in the skeleton. Using that approach, Day *et al* showed that addition of calcium lactate to infant milk feeds resulted in calcium retention equal to that attained in utero[16]; however, in their study ionic hypercalcaemia occurred in over half the treated patients and the disparity between calcium retention (160 mg/kg/day) and phosphorus retention (25 mg/kg/day) remains unexplained. A similar disparity is present in the results of a study performed by Moya and Domenech that was probably too short (three days) to provide a reliable estimate of longer term calcium retention.[17] More recent studies, in which specially prepared feeds were used that are not routinely available, have shown that mineral supplementation of formula feeds may result in calcium retention comparable with that achieved in utero.[18 19] Further evidence of the benefits of mineral supplementation has been provided by radiographic studies which showed fewer abnormal wrist radiographs in infants receiving mineral supplemented formula feed.[20]

From a technical standpoint, it is preferable to measure changes in skeletal mineralisation using photon absorptiometry, rather than calcium balance or radiological techniques, because the method provides a precise and accurate direct measurement of the bone mineral content at the scan site.[1 2] Absorptiometric data on the effect of human milk feeding on changes in bone mineral content are confusing. A significant fall in bone mineral content during human milk feeding has been shown,[21 22] but a significant fall has been shown in formula fed infants too.[22] The addition of mineral supplements to human milk feeds has been found to confer no extra advantage in promoting bone mineral accretion.[23]

The first workers to report normalised rates of bone mineralisation, based on absorptiometric data, in preterm infants given supplemented feeds were Steichen and Tsang.[6] Unlike the infants in our study, theirs received not only mineral but also vitamin D supplements; furthermore the groups were not accurately described and the control group unexpectedly had a significantly lower mean value of bone mineral content at 2 weeks of age than the supplemented group despite the fact that both received the same feed for the first six days of life. However, there is evidence that the very smallest infants do not do so well. A further study by the same group, in which the benefits of mineral and vitamin D supplementation in infants weighing less than 1500 g were investigated, showed that in two thirds of the infants bone mineral content increased, while in one third of the cases bone mineral content fell.[24] Chan *et al* studied mineral supplementation in carefully selected infants who weighed less than 1600 g; they excluded from their study infants who were ventilated, those who received more than 50% ambient oxygen, those with sepsis or intraventricular haemorrhage and those who received total parenteral nutrition for more than five days.[21] Thus infants most at risk of osteopenia due to nutritional compromise were excluded. None the less, in that study also, some infants appeared to respond to supplementation by increasing bone mineral content whereas others suffered a net loss of bone mineral. A more recent study using very high concentrations of mineral in formula feed (180 mg/100 ml calcium and 90 mg/100 ml phosphorus) showed a significant increase in bone mineral content, but the increase did not match the change expected in utero.[22] The risk of mineral precipitation at such concentrations is quite high and supplementation to this level is well above present guidelines.[25] At the much lower concentrations used in our study, we found no evidence of mineral precipitation.

The selection of infants at reduced risk of osteopenia, the use of complicated formula feeds, and the concomitant use of vitamin D as well as mineral supplements, has made the interpretation of the results of previous studies and the choice of an optimal dietary regime in preterm infants difficult for the practising paediatrician. With this in mind, in our study we regarded all infants as eligible for study no matter how ill they were, we used a simple method of mineral supplementation using standard cows' milk formula, and we used no vitamin D supplement over and above the standard supplement of 400 IU/day. Furthermore, no attempt was made to increase mineral accretion rates to the values achieved in utero because sick preterm infants often do not match intrauterine rates of growth in weight and length.

Under those conditions, we have shown that mineral supplementation of formula feeds confers a small but significant benefit in terms of mineral accretion rate, with rates of weight and length increase being independent of the dietary regimes studied. No side effects such as acidosis[26] and necrotising enterocolitis[27] occurred in the supplemented group. Although fat malabsorption has previously been shown in infants given mineral supplemented milk,[28 29] there was no evidence of reduced weight gain in our supplemented infants. We therefore conclude that mineral supplementation of formula feed at the levels used in our study is a worthwhile and safe approach to the reduction of ostepenia of prematurity in all infants whose mothers choose not to breast feed.

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References

[1] James JR, Truscott J, Congdon PJ, Horsman A. Measurement of bone mineral content in the human fetus by photon absorptiometry. *Early Hum Dev* 1986;**13**:169–81.

[2] James JR, Congdon PJ, Truscott J, Horsman A, Arthur R. Osteopenia of prematurity. *Arch Dis Child* 1986;**61**:871–6.

[3] Horsman A, Ryan SW, Congdon PJ, Truscott JG, James JR. Osteopenia in extremely low birthweight infants. *Arch Dis Child* 1989;**64**:485–8.

[4] Hillman LS, Hollis B, Salmons S, *et al*. Absorption, dosage and effect on mineral homeostasis of 25-hydroxycholecalciferol in premature infants. Comparison with 400 and 800 IU vitamin D_2 supplementation. *J Pediatr* 1985;**106**:981–9.

[5] Ryan SW, Congdon PJ, James JR, Truscott J, Horsman A. Mineral accretion in the human fetus. *Arch Dis Child* 1988;**63**:799–808.

[6] Steichen JJ, Tsang RC. Osteopenia of prematurity; the cause and possible treatment. *J Pediatr* 1980;**96**:249–52.

[7] Parkin JM, Hey EN, Clowes JS. Rapid assessment of gestational age at birth. *Arch Dis Child* 1976;**51**:259–63.

[8] Hittner HM, Hirsch NJ, Rudolph AJ. Assessment of gestational age by examination of the anterior vascular capsule of the lens. *J Pediatr* 1977;**91**:455–8.

[9] Sagy M, Birenbaum E. Phosphate depletion syndrome in a premature infant fed human milk. *J Pediatr* 1980;**96**:683–5.

[10] Lyon AJ, McIntosh N. Calcium and phosphorus balance in extremely low birthweight infants in the first six weeks of life. *Arch Dis Child* 1984;**59**:1145–50.

[11] Schanler RJ, Garza C, O'Brian Smith E. Fortified mothers' milk for very low birth weight infants: results of macromineral balance studies. *J Pediatr* 1985;**107**:767–74.

[12] Lachman E. Osteoporosis: potentialities and limitations of its roentgenologic diagnosis. *AJR* 1955;**74**:712–8.

[13] Lyon AJ, McIntosh N, Wheeler K, Brooke OG. Hypercalcaemia in extremely low birthweight infants. *Arch Dis Child* 1984;**59**: 1141–4.

[14] Rowe J, Rowe D, Horak E, *et al*. Hypophosphataemia and hypercalcuria in small premature infants fed human milk: evidence for inadequate dietary phosphorus. *J Pediatr* 1984;**104**:112–7.

[15] Schanler RJ, Abrams SA, Garza C. Bioavailability of calcium and phosphorus in human milk fortifiers and formula for very low birth weight infants. *J Pediatr* 1988;**113**:95–100.

[16] Day GM, Chance GW, Radde IC, Reilly J, Park E, Sheepers J. Growth and mineral metabolism in very low birthweight infants. II Effects of calcium supplementation on growth and divalent cations. *Pediatr Res* 1975;**9**:568–75.

[17] Moya M, Domenech E. Role of calcium-phosphate ratio of milk formulae on calcium balance in low birth weight infants during the first three days of life. *Pediatr Res* 1982;**16**:675–81.

[18] Rowe JC, Goetz CA, Carty DE, Horak E. Achievement of in utero retention of calcium and phosphorus accompanied by high calcium excretion in very low birth weight infants fed a fortified formula. *J Pediatr* 1987;**110**:581–5.

[19] Giles MM, Featon MH, Shaw B, *et al*. Sequential calcium and phosphorus balance studies in preterm infants. *J Pediatr* 1987;**110**:591–8.

[20] Laing IA, Glass EJ, Hendry GMA, *et al*. Rickets of prematurity: calcium and phosphorus supplementation. *J Pediatr* 1985;**106**:265–8.

[21] Chan GM, Mileur L, Hansen JW. Effects of increased calcium and phosphorus formulas and human milk on bone mineralisation in preterm infants. *J Pediatr Gastroenterol Nutr* 1986;**5**:444–9.

[22] Greer FR, McCormick A. Improved bone mineralization and growth in premature infants fed fortified own mother's milk. *J Pediatr* 1988;**112**:961–9.

[23] Gross SJ. Bone mineralization in preterm infants fed human milk with and without mineral supplementation. *J Pediatr* 1987;**111**:450–8.

[24] Greer FR, Steichen J, Tsang RC. Effects of increased calcium, phosphorus and vitamin D intake on bone mineralization in very-low-birth-weight infants fed formulas with polycose and medium-chain triglycerides. *J Pediatr* 1982;**100**:951–5.

[25] Bremer HJ, Brooke OG, Orzalesi M, *et al*. Nutrition and feeding of preterm infants. *Acta Paediatr Scand* 1987;**336** (suppl): 2–14.

[26] Kildeberg P, Engel K, Winters RW. Balance of net acid in growing infants: Endogenous and transintestinal aspects. *Acta Paediatr Scand* 1969;**58**:321–9.

[27] Herbst JJ. Diet and necrotising enterocolitis. In: Moore TD, ed. *Necrotising enterocolitis in the newborn infant*. 68th Ross Conference on Pediatric Research. Columbus, Ohio: Ross Laboratories, 1975:71–4.

[28] Katz L, Hamilton JR. Fat absorption in infants of birthweight less than 1,300 gm. *J Pediatr* 1974;**85**:608–14.

[29] Chappell JE, Clandinin MT, Kearney-Volpe C, Reichman B, Swyer PW. Fatty acid balance studies in premature infants fed human milk or formula: effect of calcium supplementation. *J Pediatr* 1986;**108**:439–47.

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Bone mineralisation in preterm infants measured by dual energy radiographic densitometry

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SUMMARY Dual energy radiographic densitometry was used to follow postnatal changes in the bone mineral content of the radii of 15 infants of less than 30 weeks' gestation. The system permitted bone mineral content to be measured with minimal disturbance to the infants in their incubators. Mean bone mineral content at birth was 2·4 mg/mm shaft length, decreasing to 1·9 mg/mm at 6 weeks of age, before starting to rise. Mineralisation was poor compared with that of a fetus at an equivalent postconceptual age. Mean intakes of calcium and phosphate were considerably less than the intrauterine accumulation of these minerals and it is postulated that this was the main cause of the poor mineralisation. Radiographic densitometry is both accurate and precise and has advantages over photon absorptiometry in that it can be used to measure bone mineral in infants who are not only preterm, but also ill enough to require intensive care.

The bones of a preterm infant are shorter, lighter, and contain less calcium/unit length than those of a fetus at an equivalent gestational age.[1] Osteopenia and rachitic changes are frequent radiographic findings, and spontaneous fractures are common. Biochemical abnormalities, particularly hypercalcaemia, hypophosphataemia, and increased alkaline phosphatase activity, have been reported in association with problems of bone mineralisation in premature babies.[2]

Identifiable radiographic changes are late manifestations of the metabolic bone disease of prematurity and are non-specific. To study this problem further a method for measuring bone mineral content is needed. Photon absorptiometry is both sensitive and precise and has been used by several workers.[3 4] The equipment is, however, expensive and its bulk makes it impractical for measurements on babies who are too ill to be removed from their incubators.

We have developed a system for measuring bone mineral content in preterm infants using dual energy radiographic densitometry that can be used on preterm infants while they are undergoing intensive care.[5]

The aim of this study was to use this technique to measure and follow the postnatal changes in bone mineral content of the radii of preterm infants.

Patients and methods

Infants of less than 30 weeks' gestation were studied. Informed consent was obtained from the parents.

Dual energy radiographs at 40 kv and 120 kv were taken of the forearm within three days of birth, at 7 days of age, and then weekly until discharge from the unit. The arm was held in a plastic holder that incorporated a calibration wedge with steps of aluminium and perspex of varying thicknesses. The radiographs were taken with the mobile camera routinely used on the neonatal unit and with minimal disturbance to the infants in their incubators.

Records were kept of the intravenous and oral intakes of calcium and phosphate, but in this study no changes were made to the feeding policy of the unit. Weight was recorded weekly and the length of each radius measured directly from the radiographs. The films were digitised by computer. The effect of soft tissue was subtracted out by the use of low and high energy films. Comparison with the step wedge allowed the image of the radius to be converted into an equivalent mass of aluminium and perspex. The mineral content of the radius expressed as mg of calcium and phosphorus/mm shaft length was then calculated using known values for the radiographic

Table *Details of 15 infants. Values are expressed as mean (range)*

Gestation (weeks)	*Birth weight (g)*	*Period studied (weeks)*
27·3 (25–29)	1133 (820–1480)	9 (5–13)

mass attentuation coefficients of aluminium, perspex, and bone.[5]

The total mineral content of the distal half of each radius was measured and from this the average bone mineral content/mm shaft length was obtained. Changes in the mineral content of the metaphysis of each bone were also measured at a point 2 mm from the distal end.

Results

Fifteen infants were studied (table), and bone mineral content varied along the shaft of the radius (fig 1). On all radiographs there was a dense band at the distal end of the bone and, for measurement purposes, this first peak (A) was taken as marking the start of the bone. Proximal to this peak there was an area of lower density (B), and this region showed the greatest changes in mineral content with postnatal age.

Mineral content measured at birth increased with increasing gestational age (fig 2). This figure also includes data from four infants of gestational ages of 30 weeks or more. The values lie close to the curve published by Greer *et al* using photon absorptiometry,[6] which shows the mean bone mineral content of newborn infants at various gestational ages. At the lower gestational ages the measurements from this study are lower than those of Greer *et al*, but all the points lie within two standard deviations of the published mean values.

In figs 3 and 4 it can be seen that the bone mineral content of the metaphysis and the average mineral content of the shaft of the radius changed with postnatal age. In each figure the changes are compared with an 'intrauterine' standard. This has been calculated by taking an 'average' infant from the group studied (that is, 27 weeks as shown in the

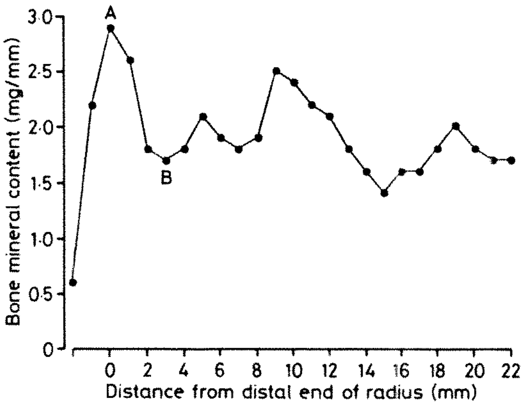

Fig 1 *Plot of bone mineral content (mg/mm) along distal half of radius. A=first peak; B=area of lower density.*

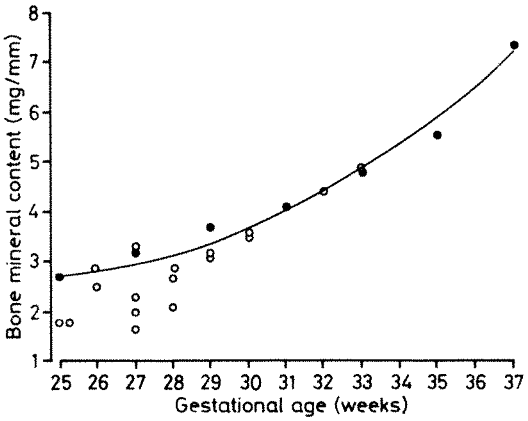

Fig 2 *Bone mineral content of individual infants at birth plotted against gestational age (open circles) compared with data obtained by photon absorptiometry (closed circles).*[6]

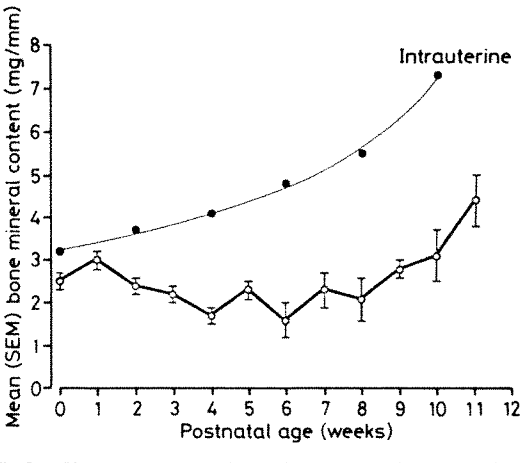

Fig 3 *Changes in mean (SEM) bone mineral content of the metaphysis with postnatal age. Expected fetal mineralisation shown in upper trace.*

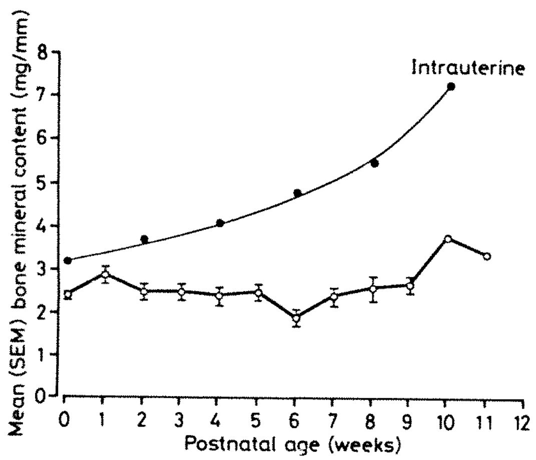

Fig 4 *Changes in mean (SEM) bone mineral content of shaft of radius with postnatal age. Expected fetal mineralisation shown in upper trace.*

table), and, using the data of Greer *et al*, plotting what would have happened to its bone mineralisation had it remained in the uterus. It can be seen that had the infants remained in utero, mineralisation would have been significantly greater than was achieved after birth.

The data for the first six weeks of life in figs 3 and 4 are similar. At birth the mean (SEM) bone mineral content was 2·4 (0·1) mg/mm, rising to 3·0 (0·2) mg/mm by 6 days of age. There was then a fall to a minimum of 1·9 (0·2) mg/mm at 6 weeks, followed by a steady rise in the mineral content of the metaphysis (fig 3). The average bone mineral content of the shaft also increased after 6 weeks, but the rise was less pronounced than that seen in the metaphysis (fig 4).

Despite the apparent poor mineralisation the infants continued to gain weight and increase bone length (fig 5). Birth weight was regained by 10 to 14 days, followed by a mean weight gain of 14 mg/kg/day (similar to that of the fetus). From birth the radius increased in length by about 1·5 mm/week.

Using an 'average' infant from the group studied, the data in fig 6 show the expected accumulation of minerals had it remained in the uterus[7] and this is compared with the actual intakes of calcium and phosphate plotted against postnatal age. Not all the

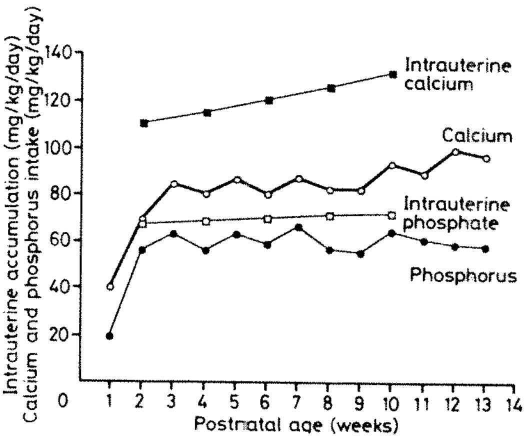

Fig 6 *Mean intakes of calcium and phosphate plotted against postnatal age and compared with calculated intrauterine accumulations.*[7]

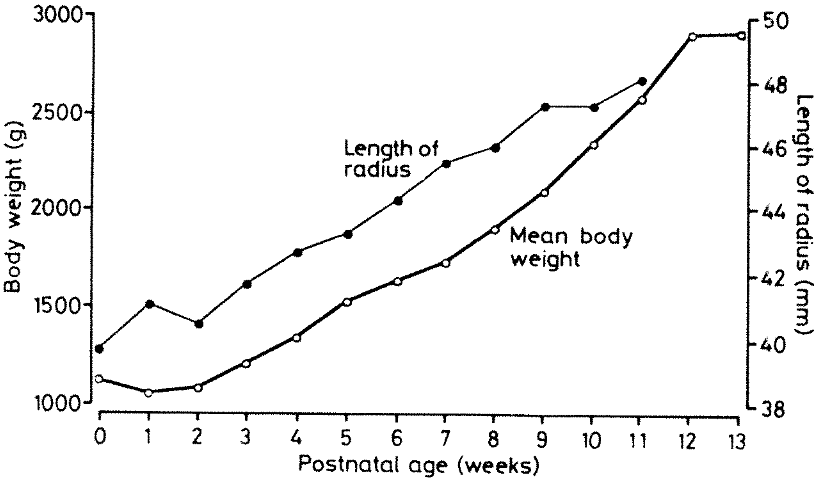

Fig 5 *Change in mean body weight and length of radius with postnatal age.*

dietary mineral would be retained, so the actual accretion would be significantly less than the intakes shown in the figure and much lower than the amounts the infant would have received if it had still been in the uterus.

No changes were made to the feeding policy of the unit. Preterm infants were initially fed with expressed breast milk or parenteral nutrition if oral feeds were not tolerated. Once feeding was established, expressed breast milk or a preterm formula was used. Mineral supplements were added to the breast milk at the discretion of the medical staff. All infants were given 1000 units of vitamin D a day. There was no correlation between intake and mineral content of the radius.

Discussion

The use of dual energy radiographic densitometry has permitted the measurement of bone mineral in preterm infants, even when they are sick and undergoing intensive care. The film, holder, and calibration wedge fit easily into an incubator and the baby needs the minimum of handling. Radiation dosage is low, a whole body equivalent of 0·4 μSv compared with 17·4 μSv for a chest radiograph.[5]

Using photon absorptiometry, mineral content is measured in a short segment of bone.[3] [4] The data in fig 1, however, show that mineral content is not uniform along the shaft of the bone. In longitudinal studies it is important to scan the same segment of bone on each occasion, and this may be difficult to locate using skin surface markings. Data in figs 3 and 4 show that changes in one part of the bone may not reflect what is happening in the bone as a whole. Changes in mineral content of the metaphysis occurred earlier and were more pronounced than in the rest of the bone. This is perhaps not surprising as the metaphysis is the most active part of the bone.

A radiograph easily relates measurement to a fixed position on the bone. It is also possible to measure mineral at various points on the bone, or to calculate the total bone mineral content. Any bone may be used as long as it can be immobilised to allow two radiographs to be taken with the bone in the same position. In this study the distal half of the radius was used but with minor alterations to the technique it would be possible to measure total mineral content in both forearm bones, which will reduce the effect of variations along a bone or between different bones.

The data in fig 2 show that there is reasonable correlation between bone mineral measurements obtained using radiographic densitometry and those from photon absorptiometry, and this supports the other validation studies of the system.[5] At the lower gestational ages, however, the results from this study appear to be consistently below those published by Greer *et al*. This may be due to differences in the racial composition of the two populations. In the study of Greer *et al* the patients were predominantly white (92%), but in this study eight were white and seven Asian. At less than 35 weeks' gestation Asian infants have lower bone mineral contents than white babies,[8] and this may have influenced the results in this study.

During the first week of life there was a rise in bone mineral content in every baby studied. During the first few days of life preterm infants showed a fall in plasma calcium concentration, possibly due to low parathyroid hormone and to high circulating concentrations of calcitonin.[9] The initial rise in mineral content could well be a result of these hormonal changes. Over the next 4 to 6 weeks of life the bone mineral content decreased. The bones continued to increase in length, suggesting that matrix was being laid down even if bone mineralisation was poor.

The data in fig 5 show that these babies had mineral intakes considerably lower than a fetus of equivalent postconceptual age, and this was probably the main reason for the poor mineralisation. After 6 weeks bone mineral content increased even though there was no change in dietary intake of calcium and phosphate. This may have been due to an improvement in the general health of the baby or to increased retention of mineral as a result of better gut absorption and lower renal losses.[10] [11]

The fact that bone mineral content improved spontaneously does not mean that a period of suboptimal mineralisation is acceptable. The long term consequences are not known. Dental defects have been associated with neonatal hypocalcaemia and it has been postulated that transient disturbances in calcium metabolism at a sensitive period of tooth formation can have serious effects on enamal calcification.[12] In the short term, better mineralisation should reduce the chances of spontaneous fractures and lessen the possibility of rachitic respiratory distress.[13]

Nutrition groups in America[14] and Europe[15] now recommend intakes of calcium of 180–210 mg/kg/day and phosphate of 120–140 mg/kg/day, which are far in excess of the intakes given in this study and greater than those given in most neonatal units in Britain. There is some evidence that in preterm infants who are otherwise well these higher intakes result in better bone mineralisation.[16]

There may, however, be a problem in improving mineral retention in sick preterm babies who need intensive care. These infants are often parenterally fed for long periods and it is difficult to keep high concentrations of calcium and phosphate in solution. Problems such as patent ductus arteriosus are often managed by fluid restriction, with subsequent reduction in the intakes of all nutrients including calcium and phosphorus.

Dual energy radiographic densitometry is a convenient method for accurately measuring and following changes in the bone mineral content of preterm infants. It entails minimal handling and will be used in further studies to measure the effect of diet on the mineralisation of those infants who are not only preterm but also ill enough to require intensive care.

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References

[1] McIntosh N, Shaw JCL, Taghizadeh A. Direct evidence for calcium and trace mineral deficits in the skeleton of preterm infants. *Pediatr Res* 1974;**8**:896.

[2] Lyon AJ, McIntosh N, Wheeler K, Brooke OG. Hypercalcaemia in extremely low birthweight infants. *Arch Dis Child* 1984;**59**: 1141–50.

[3] James JR, Truscott J, Congdon PJ, Horsman A. Measurement of bone mineral content in the human fetus by photon absorptiometry. *Early Hum Dev* 1986;**13**:169–81.

[4] Greer FR, Lane J, Weiner S, Mazess RB. An accurate and reproducible absorptiometric technique for determining bone mineral content in newborn infants. *Pediatr Res* 1983;**17**:259–62.

[5] Hawkes DJ, Lyon AJ, Doran M, McIntosh N. A technique to measure the bone mineralisation of preterm infants using digitised dual energy radiographs. *Med Phys* (in press).

[6] Greer FR, McCormack A. Bone growth with low mineral content in very low birthweight premature infants. *Pediatr Res* 1986;**20**:925–8.

[7] Shaw JCL. Malnutrition in very low birthweight, preterm infants. *Proc Nutr Soc* 1974;**33**:103–11.

[8] Ryan S, Congdon PJ, James J, Truscott J, Horsman A. Mineral accretion in the human fetus. *Arch Dis Child* 1988;**63**:799–808.

[9] Romagnoli C, Zecca E, Tortorolo G, Diodata A, Fazzini G, Sorcini-Carta M. Plasma thyrocalcitonin and parathyroid hormone concentrations in early neonatal hypocalcaemia. *Arch Dis Child* 1987;**62**:580–4.

[10] Shaw JCL. Evidence for defective skeletal mineralisation in low birthweight infants: the absorption of calcium and fat. *Pediatrics* 1976;**57**:16–25.

[11] Lyon AJ, McIntosh N. Calcium and phosphorus balance in extremely low birthweight infants in the first six weeks of life. *Arch Dis Child* 1984;**59**:1145–50.

[12] Stimmler L, Snodgrass GJAI, Jaffe E. Dental defects associated with neonatal symptomatic hypocalcaemia. *Arch Dis Child* 1973;**48**:217–20.

[13] Glasgow JFT, Thomas PS. Rachitic respiratory distress in small premature infants. *Arch Dis Child* 1977;**52**:268–74.

[14] American Academy of Pediatrics: Committee on Nutrition. Nutritional needs of low-birthweight infants. *Pediatrics* 1985;**75**: 976–86.

[15] ESPGAN Committee on Nutrition. Nutrition and feeding of preterm infants. *Acta Paediatr Scand* 1987;**336**(suppl):9–10.

[16] Chan GM, Mileur L, Hansen JW. Effects of increased calcium and phosphorus formulas and human milk on bone mineralisation in preterm infants. *J Pediatr Gastroenterol Nutr* 1986;**5**:444–9.

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Limitations of patient triggered ventilation in neonates

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SUMMARY The practicality of long term patient triggered ventilation using airflow changes was assessed in 22 infants with a median gestational age of 29 weeks (range 25–33 weeks). Inflation time during patient triggered ventilation was limited to 0·4 seconds or less. Initially it was associated with improvements in oxygenation in most infants. Patient triggered ventilation was maintained till final extubation in 13 of the infants without complications. Only one infant developed a pneumothorax, but in the remaining eight infants (who tended to be less mature ($p<0·01$)) it had to be discontinued after only a few hours. Predictors of failure of patient triggered ventilation at one hour were both a lack of improvement in oxygenation, and a relatively slow triggering rate that was related to gestational age. We conclude that long term patient triggered ventilation is practical for preterm neonates, but only for those more mature than 28 weeks' gestational age.

Patient triggered ventilation has been used in preterm infants with success.[1–3] A variety of triggering mechanisms have been used including detection of inspiratory flow changes at the airway, which has been shown to be reliable even in infants of very low birth weight.[3]

Change to patient triggered ventilation from conventional mode has been associated with immediate improvements in oxygenation, particularly during the recovery phase of respiratory distress syndrome.[3] In those preliminary studies that investigated the feasibility of patient triggered ventilation in the very preterm neonate it was maintained for short periods only. The aim of this study was to assess the practicality of long term patient triggered ventilation in preterm neonates and to examine possible limitations.

Patients and methods

Twenty two infants, with a median gestational age of 29 weeks (range 25–33) and birth weight of 1450 g (range 560–1910) were entered into the study. Their median postnatal age was 30 hours (range 3–120). All infants included in the study were being ventilated because of respiratory distress syndrome or respiratory distress associated with severe prematurity. Most of the infants had been ventilated from birth, but some more mature babies, all with respiratory distress syndrome, had been ventilated because of worsening blood gas tensions.[4] Ventilator settings before the study were determined by the clinical team caring for the infants. Ventilator rates, except in three infants who were more than 72 hours old, were in excess of 60 breaths/minute. No infant was paralysed or had received sedation.

The 22 infants represented those patients ventilated consecutively with the SLE Newborn 250 ventilator in patient trigger mode. Patient triggered ventilation was started during the acute stage of the infant's illness according to the availability of the ventilator (fig 1).

Infants were entered into the study once they had been stable on conventional ventilation for at least two hours. They were then transferred to continuous positive airways pressure (CPAP) trigger mode. The function of the SLE ventilator in patient triggered mode and the pneumotachograph triggering sensor have previously been described in detail.[3] Briefly, the pneumotachograph, placed between the endotracheal tube and the ventilator circuit, detects changes in flow and triggering occurs if the inspiratory flow exceeds 0·4 l/minute. If respiratory efforts are insufficient to cause triggering after seven seconds, a manual breath will be automatically delivered.

The inspired oxygen concentration, peak inspiratory pressure and positive end expiratory pressure (PEEP) were not altered on the introduction of the patient trigger mode. A standard flow rate within

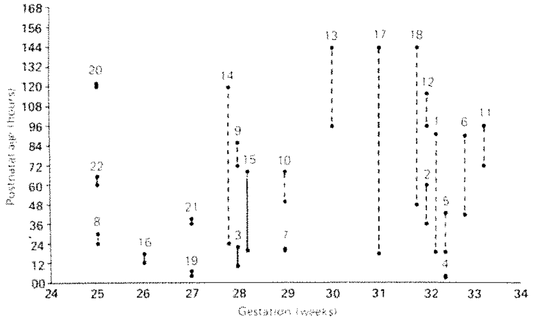

Fig 1 *The postnatal and gestational ages of the infants receiving patient triggered ventilation. Each infant is represented by two linked data points, the length of the joining line representing the duration that the ventilation was maintained. Infants in whom it was successfully maintained until extubation are depicted by the interrupted lines, the failures by the solid lines.*

the ventilator circuit was used in all infants, 8l/minute, and this was kept constant during patient triggered ventilation. The inflation time was altered after observation of the infant's respiratory rate while receiving CPAP for one minute.[5] From the respiratory rate the inspiratory time was calculated to yield an inspiratory:expiratory (I:E) ratio of 1:1·2. As a systems delay of up to 40 milliseconds can be experienced,[3] the inflation time set during patient triggered ventilation was the calculated inspiratory time minus 40 milliseconds.

On starting patient trigger mode the infant's respiratory efforts during ventilation were observed. If the inspiratory efforts were distinct from inflation, evidence of active expiration or asynchrony,[6] the duration of inflation was reduced in steps to a minimum of 0·1 seconds. Detailed respiratory measurements were then made, simultaneously recording flow, volume, and oesophageal and ventilator pressure changes as previously described.[6] Infants who, despite reduction in inflation time, remained asynchronous were returned to conventional mode.

Throughout the period of ventilation the infants were continuously monitored using either a Searle intra-arterial electrode or a transcutaneous oxygen monitor (Draeger). Arterial blood gases were sampled from an indwelling umbilical or peripheral arterial catheter. Gases were sampled immediately before and 20 minutes and one hour after, switching to trigger mode. The inspired oxygen concentration was altered to maintain arterial oxygen pressure (PaO_2) between 6·67–9·33 kPa (50–70 mm Hg). Subsequently arterial blood gases were checked at four hourly intervals, or more frequently if clinically indicated. Either peak inspiratory pressure or inspired oxygen concentration, or both, were reduced in response to improvements.

Patient triggered ventilation was continued until either ventilatory support was no longer required and the infant extubated or patient triggered ventilation 'failed'. It was considered to have failed if: the infant developed a respiratory acidosis (pH <7·25); a metabolic acidosis developed, suggesting fatigue (other causes such as hypothermia, hypovolaemia, and sepsis having been excluded); or the infant became either asynchronous despite reduction in inspiratory time, or developed a pneumothorax.

To determine the cause of the deterioration of blood gases the infant was returned to conventional mode with the pneumotachograph remaining in the airway, and arterial blood gases were estimated 20 minutes later. Improvement indicated that inconsistent triggering had been responsible, no improvement suggested that the original deterioration was a result of the added dead space of the pneumotachograph within the airway. The latter was confirmed if the blood gases improved after a further period on conventional mode with the pneumotachograph removed from the airway.

Inspired oxygen concentrations and rate of delivery of positive pressure inflations during conventional mode were compared with those after one hour on patient triggered ventilation. Differences were assessed for significance by the paired Wilcoxon rank sum test. Comparisons were also made between those infants in whom patient triggered ventilation failed, and those in whom it succeeded. Differences between the groups were statistically assessed by the Wilcoxon rank sum test and Fisher's exact test. This study was approved by the hospital's ethics committee.

Results

Patient triggered ventilation was maintained for variable times in the 22 infants. The median duration was 19 hours (range 1–240). In all 22 infants the inflation time used was 0·4 seconds or less and in the more immature infants it was as low as 0·2 seconds, median inflation time being 0·32 seconds. Initially all infants were synchronous as determined by clinical observation.[6] Those in whom long term patient triggered ventilation was successful remained synchronous without further change in the inflation time.

One infant (of 32 weeks' gestation) developed a serious complication during patient triggered ventilation—a pneumothorax—12 hours after starting the trigger mode. The infant became

Table *Inflation time and pressure, gestational age, and changes in inspired oxygen concentrations*

Inflation time (secs)	*Peak inspiratory pressure (cm H_2O)*	*Gestational age (weeks)*	*FiO_2 before ventilation (%)*	*FiO_2 one hour after ventilation (%)*
Infants for whom patient triggered ventilation was successful:				
0·4	24	32	60	60
0·35	18	32	45	40
0·35	24	32	70	60
0·4	22	33	50	45
0·4	15	25	30	25
0·3	16	28	30	25
0·27	17	29	40	30
0·4	21	33	40	35
0·4	18	32	40	35
0·28	17	30	65	55
0·3	14	28	55	45
0·32	26	31	50	45
0·36	24	32	50	45
Infants for whom patient triggered ventilation failed:				
0·27	16	28	60	40
0·4	24	32	85	95
0·25	24	29	60	60
0·32	32	28	65	75
0·25	15	26	30	21
0·31	18	27	80	80
0·2	24	25	70	75
0·33	19	27	50	40
0·29	18	25	45	35

asynchronous immediately before the development of the airleak. A fault had developed in the expiratory line of the ventilator so that it was impossible to reduce the PEEP below 6 cm H_2O.

Comparison of arterial blood gases after one hour on patient triggered ventilation with those taken immediately before changing from conventional mode showed an improvement in oxygenation ($p<0·05$) in most of the infants. Consequently it was possible to reduce the inspired oxygen concentrations from a median of 50% (range 30–85) to 45% (range 21–95) ($p<0·05$), (table), and maintain arterial oxygen saturation within the desired range. The improvement in oxygenation was also associated with an increase in delivery rate of positive pressure inflations from a median of 60 breaths/minute (range 20–80) to 72 breaths/minute (48–120), ($p<0·01$).

Thirteen infants tolerated patient triggered ventilation until final extubation without complications. The median duration was 24 hours (range 6–240). In the remaining nine babies patient triggered ventilation failed after variable time periods (median duration 3 hours, range 1–48) (fig 1). There was no significant difference between these two groups in the postnatal age at which they started patient triggered ventilation (median 42 hours, range 18–96, and median 20 hours, range 3–120 hours, respectively). The first group, however, were significantly more mature (median 32 weeks, range 28–33) and heavier at birth (median 1600 g, range 650–1910) than the second group (median 27 weeks, range 25–32; median 1080 g, range 560–1910) ($p<0·01$). The second group also tended to receive shorter inflation times (median 0·29 secs, range 0·2–0·33) than the first (median 0·35 secs, range 0·27–0·4), ($p<0·05$).

The change to patient triggered ventilation was more often associated with a lack of improvement in oxygenation after the first hour in those infants in whom it finally failed ($p<0·05$). The inflation rate during patient triggered ventilation tended to be inversely proportional to gestational age in those infants in whom it was successful, and similar to their previously observed spontaneous respiratory rate (fig 2). In six of the infants in whom it failed (all of 28 weeks' gestation or less) the delivery rate of inflations at one hour was at least 20 breaths/minute lower than their spontaneous respiratory rate (fig 2).

Two infants whose spontaneous respiratory rates and initial triggering rate were in excess of 100/minute (fig 2) subsequently failed to trigger the ventilator on about one breath in four. These infants then became asynchronous, and were unresponsive to a reduction in inspiratory time. One infant suddenly failed to trigger the ventilator without

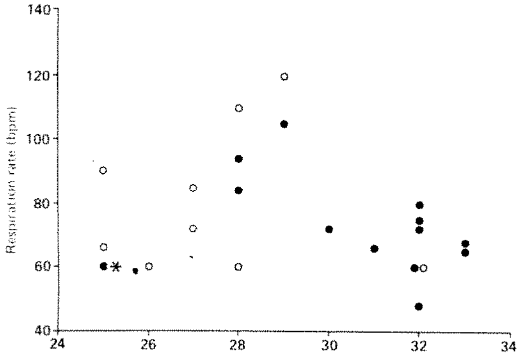

Fig 2 *The rate of delivery of positive pressure inflations at one hour related to gestational age during patient triggered ventilation.* ●=*those for whom it was successful;* ○=*those for whom it failed;* *=*an infant of 25 weeks' gestation in whom patient triggered ventilation was only started during weaning.*

obvious cause. The remaining five infants developed a metabolic acidosis after variable periods on patient triggered ventilation. In all cases this reversed after the infant returned to conventional mode, despite the pneumotachograph remaining in the circuit.

Discussion

This study confirmed that patient triggered ventilation, using airflow changes, was beneficial in most patients. Initially all the infants, on clinical observation, became synchronous[6] and this explains the improvement in oxygenation.[7] Those infants in whom long term patient triggered ventilation was successful remained synchronous throughout ventilation and thus did not require sedation or paralysing agents.[8] Conversion to synchronous respiration and reduction in active expiration is also associated with high frequency positive pressure ventilation (HFPPV).[9] A randomised trial is at present being conducted to assess which of these two modes of ventilation is the more efficacious at preventing this respiratory interaction.

Patient triggered ventilation is successful if the infant is synchronous with the ventilator, that is if the start of positive pressure inflation occurs early in inspiration[5] and if inflation has ceased before late expiration.[10] Such timing is dependent on three factors; the duration of the systems delay, the stage during inspiration at which the infant's airflow exceeds the triggering point, and the duration of inflation. The maximum systems delay time of the SLE and pneumotachograph sensor has been shown to be 40 milliseconds. This systems delay time must be subtracted from the calculated inflation time to prevent inflation continuing into expiration. Although in the present study this meant that in some of the more immature infants the inflation time was as short as 0·2 seconds, oxygenation was maintained. A longer systems delay time, however, would certainly be a disadvantage.[11] In the present study, as seen previously,[3] the infants were able to generate the critical change in airflow early in inspiration. This success may be explained as airflow changes tend to be fastest early in inspiration. The infants included in this study all had respiratory distress and were less than one week old, thus airway resistance was likely to be low, which would be expected to facilitate early rapid airflow changes. The duration of inflation time is of crucial importance and must be sufficiently short to prevent inflation persisting into spontaneous expiration.[10] In this study this was successfully determined from clinical observation of the infants' spontaneous respiratory rates, and as a consequence, in no infant was an inflation time greater than 0·4 seconds used. This could be predicted from previous studies, which have shown that very preterm infants have a fast spontaneous respiratory rate and hence short inspiration.[5] Even during weaning, limitation of inspiratory time to 0·5 seconds improves oxygenation and prevents active expiration.[12]

Only one infant developed a pneumothorax during patient triggered ventilation; this low incidence may, however, simply reflect the population studied, and a randomised trial is necessary before it can be claimed that patient triggered ventilation is associated with a reduction in the incidence of air leaks. Although the infant who developed an airleak had become asynchronous immediately before the pneumothorax developed, a simultaneous increase in PEEP was noted, a factor previously incriminated in increasing the incidence of airleaks.[13] On examination of the ventilator a malfunction of the expiratory valve was noted and this was independent of ventilator mode.

This study has outlined important limitations of patient triggered ventilation among preterm neonates. Disappointingly, in addition to the infant who developed a pneumothorax, patient triggered ventilation was considered to have failed on a further eight occasions. It failed in the more immature infants of less than 28 weeks' gestational age. The only infant of less than 28 weeks' gestation who tolerated patient triggered ventilation till extubation was only started on it during weaning (conventional ventilator rate at 20 breaths/minute), and thus was recovering from respiratory distress.

Failure of patient triggered ventilation was seen in two infants whose triggered inflation rates exceeded 100 breaths/minute. Both infants subsequently failed to generate sufficient airflow change to trigger the ventilator consistently and they then became asynchronous. At such high inflation frequencies ventilator function is impaired and, in particular, inadvertent PEEP may occur.[14] This may have contributed to the change in respiratory effort. A further problem is that until the ventilator responds to the patient's inspiratory efforts—that is, the time taken by the systems delay—the infant's respiration is essentially unsupported and the ventilator is acting not as a ventilator but as a respiratory obstruction, which became more apparent at increased rates. It has been suggested that the response time, or systems delay time, should not exceed 10% of the total inflation time; a systems delay of 0·036 seconds would permit a maximum ventilator rate of 83 breaths/minute.[11] As occurred in this study, when triggering was attempted at an appreciably higher rate than that allowed by the response time, incoordination with the ventilator resulted.[11]

Another possible explanation for the failure of patient triggered ventilation in the immature infants was the use of short inflation times in this group. Limitation of inflation time reduces the area under the pressure/time curve for a given breath and decreases the assistance delivered by the ventilator. Ventilator waveform is also affected by a reduction in inflation time,[15] particularly if—as in this study—flow is not increased. This may have compromised the effectiveness of ventilation further. Limitation of inflation time was necessary to prevent asynchrony, and short inflation times were tolerated successfully by some infants to extubation. Increasing peak inspiratory pressure would have increased the mean airway pressure and thus possibly have compensated for the limitation of inflation time. We were reluctant, however, to adopt this approach with its known hazards,[16] particularly as among nonparalysed infants short inspiratory times, without an increase in peak inspiratory pressure, can be associated with improvements in oxygenation if delivered at fast rates.[7]

Failure of patient triggered ventilation in the remaining infants was associated with the development of a metabolic acidosis. In all cases this disappeared once the infant had been returned to conventional mode, even though the pneumotachograph remained in the circuit. This suggested that the metabolic acidosis was due to fatigue. Although the added deadspace of the pneumotachograph in the airway has previously not been associated with deterioration in blood gas tensions,[17] among these tiny infants it may have added critically to the work of breathing. This airflow triggering system resulted in synchronous respiration in all infants, thus an advantageous future development would be an alternative method of detection of airflow changes that did not appreciably increase the deadspace of even the smallest infants.

The present results have shown useful early predictors of failure of patient triggered ventilation, that is a failure of improvement in oxygenation and slow triggering rate when related to gestational age. Immature infants with respiratory distress have previously been shown to have a fast spontaneous respiratory rate in the first 48 hours of life.[5] This was recorded, however, during a very short time period.[5] It seems likely that very preterm infants, particularly considering the limitations of patient triggered ventilation at fast rates noted above, may be unable to sustain such rapid rates even for one hour during patient triggered mode. The failure of improvement in oxygenation at one hour would then be consequent on the comparatively slow triggering rate.[7] Our results suggest that any infant who fails to trigger the ventilator at a similar frequency, that is within 20 breaths/minute, to his or her own respiratory rate, or in whom oxygenation fails to improve after one hour on patient triggered ventilation, should be returned to conventional mode. In this group of infants during the acute stage of the illness HFPPV may be more successful,[7] and patient triggered ventilation may be reserved for weaning.[3]

In summary, patient triggered ventilation using airflow detected at the airway as the triggering mechanism can be an effective form of long term neonatal ventilation, but there are limitations, particularly in the more immature infants. The success or failure of this mode of ventilation is usually apparent within a short time period. Randomised studies are required to determine if patient triggered ventilation, when compared with HFPPV, is advantageous to the preterm neonate in reducing the duration of mechanical ventilation or long term complications.

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References

[1] Mehta A, Callan K, Wright BM, Stacey TE. Patient triggered ventilation in the newborn. *Lancet* 1986;**ii**:706–12.

[2] Greenough A, Greenall F. Patient triggered ventilation in premature neonates. *Arch Dis Child* 1988;**63**:77–8.

[3] Greenough A, Pool J. Neonatal patient triggered ventilation. *Arch Dis Child* 1988;**63**:394–7.

[4] Greenough A, Roberton NRC. Morbidity and survival in neonates ventilated for the respiratory distress syndrome. *Br Med J* 1985;**290:**597–600.

[5] Greenough A, Greenall F, Gamsu H. Synchronous respiration, which ventilator rate is best? *Acta Paediatr Scand* 1987;**76:** 713–6.

[6] Greenough A, Greenall F. Observation of spontaneous respiratory interaction with artificial ventilation. *Arch Dis Child* 1988;**63:**168–71.

[7] Greenough A, Pool J, Greenall F, Morley C, Gamsu H. Comparison of two different rates of artificial ventilation in premature neonates with RDS. *Acta Paediatr Scand* 1987;**76:** 706–12.

[8] Greenough A, Wood S, Morley C, Davis J. Pancuronium prevents pneumothoraces in ventilated premature babies who actively expire against positive pressure inflation. *Lancet* 1984; **i:**1–4.

[9] Greenough A, Morley CJ, Pool J. Fighting the ventilator are fast rates an alternative to paralysis? *Early Hum Dev* 1986;**13:** 189–94.

[10] Greenough A. The premature infant's respiratory response to mechanical ventilation. *Early Hum Dev* 1988;**17:**1–5.

[11] Epstein RA. The sensitivities and response times of ventilatory assists. *Anesthesiology* 1971;**34:**321–6.

[12] Greenough A, Greenall F, Gamsu H. Inspiratory times when weaning from mechanical ventilation. *Arch Dis Child* 1987;**62:** 1269–70.

[13] Berg TJ, Pagtakhan RD, Reed MH, Langston C, Cherniack V. Bronchopulmonary dysplasia and lung rupture in HMD: influence of continuous distending pressure. *Pediatrics* 1976;**55:** 51–3.

[14] Greenough A, Greenall F. Performance of respirators at fast rates commonly used in neonatal intensive care units. *Pediatr Pulmonol* 1987;**3:**357–361.

[15] Boros SJ, Bind DR, Mammel KC, Kegen E, Gardo MJ. Using conventional infant ventilators at unconventional rates. *Pediatrics* 1984;**74:**487–92.

[16] Taghizadeh A, Reynolds EOR. Pathogenesis of bronchopulmonary dysplasia following hyaline membrane disease. *Am J Pathol* 1976;**82:**487–92.

[17] Greenough A, Morley CJ, Davis J. Respiratory reflexes in ventilated premature babies. *Early Hum Dev* 1983;**8:**65–75.

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In vivo distribution of human milk leucocytes after ingestion by newborn baboons

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SUMMARY The in vivo distribution of enterally administered human milk leucocytes labelled with indium hydroxyquinoline (^{111}In) was studied in premature baboons. The animals were killed at 72 hours of age and tissue samples examined for radioactivity. Maximum activity was found in the luminal contents, and activity in the liver and spleen was higher than in bone marrow, the site where free isotope is normally deposited. These findings suggest that some intact milk leucocytes may cross from the gastrointestinal tract into the neonatal circulation. Also the high activity in gastrointestinal tissue that had been washed several times indicates that leucocytes adhere to mucosa or lie intramurally. We speculate that the presence of leucocytes in the gastrointestinal tract 60 hours after a single breast feed can provide an important defence mechanism against infection.

Research continues to show the scientific benefits of breast feeding.[1] These benefits are not limited to nutritional elements alone, as breast milk contains many active immunological components including immunoglobulins and numerous cells.[2] By transfer of these factors, breast feeding has been implicated in maintaining immunological homoeostasis in the neonatal intestine during the period of immaturity of the immune system.[3-6]

Although the presence of functioning leucocytes in milk has been shown in vitro by various investigators,[7-9] their in vivo function after ingestion by the newborn has not been widely studied. There is also a lack of information about their migration pattern. The present investigation was designed to study the distribution and migration pattern of human milk leucocytes in the gastrointestinal tract of premature newborn baboons.

Material and methods

The study was approved by the Institutional Review Board and Animal Care Committee. In our laboratory a baboon fetus delivered at a mean gestational age of 140 days (normal gestation period 184 days) has been used to study hyaline membrane disease; details of the preparation of the animals have been previously described.[10] We therefore used the existing animal model to study the kinetics of human milk leucocytes.

Animals were delivered by caesarean section at a mean (SD) gestational age of 140 (2) days and were anaesthetised with ketamine. A tracheostomy was carried out for control of the airway and management of secretions. The external jugular vein was cannulated for infusion of fluids. Studies of human milk leucocytes were started when the animals were stable. Labelled cells suspended in phosphate buffered saline were given through a nasogastric tube at less than 12 hours of age. Animals received no other feeds before or after the cells were given (table 1).

ISOLATION OF MILK LEUCOCYTES

Samples of human milk were collected by manual expression from healthy mothers between two and 10 days after delivery and immediately processed using modification of the technique of Ogra and Ogra.[11] The method for the separation of the leucocytes is shown in table 2.

LABELLING OF LEUCOCYTES

The method for the labelling of the leucocytes is shown in table 3.

Blood samples were obtained through an indwelling central venous catheter at 0 and 72 hours. Animals were killed at 72 hours and tissue samples obtained from various organs. The degree of leucocyte sequestration was determined by counting the radioactivity/g of tissue and was expressed as multiples of the activity in the blood.

Table 1 *Details of study in primate model*

1 Six premature baboons, delivered at 140 days' gestation, were stabilised and maintained alive for 72 hours

↓

2 A suspension of labelled cells was fed through a nasogastric tube at <12 hours of age

↓

3 Serial samples of blood, urine, and meconium were collected at 12 hour intervals

↓

4 Animals were killed at 72 hours of age and radioactive counts performed on tissue samples obtained from liver, spleen, bone marrow, and various parts of the gastrointestinal tract

Table 2 *Isolation of milk leucocytes*

1 Human breast milk was obtained from lactating mothers <10 days' postpartum; 30 ml fresh sample was used for each experiment

↓

2 Milk was defatted by cold centrifugation at 400 *g* for 30 minutes

↓

3 Supernatant with fat layer was aspirated and fat globules removed

↓

4 Cell buttons were agitated and washed three times in phosphate buffered saline then resuspended in phosphate buffered saline

↓

5 Total and differential counts were performed and cells tested for viability with trypan blue staining

Table 3 *Labelling of leucocytes*

1 A known amount of indium hydroxyquinoline (^{111}In) was added to the suspension of washed human milk leucocytes

↓

2 The suspension was incubated at room temperature for 30 minutes

↓

3 (a) Incubated cells were centrifuged at 400 *g* for 6 minutes
(b) Supernatant was removed and labelled 'wash'
(c) Cells were resuspended in 2 ml phosphate buffered saline

↓

4 Indium hydroxyquinoline (^{111}In) activity was determined in cells and wash, and labelling efficiency calculated as follows:

$$\% = \frac{\text{Bq in labelled cells}}{(\text{Bq in cells and wash})} \times 100$$

Results

Seven animals were studied; one died on the second day of the experiment and was excluded from analysis. The four male and two female animals had a mean (SD) birth weight of 550 (110) g and gestational age 140 (2) days.

The yield of milk cells varied widely with a mean of 5 × 10^6 (2 × 10^5) cells/ml. The predominant cells were macrophages and polymorphonuclear leucocytes, with a small proportion of lymphocytes. As ^{111}In binds avidly with all blood cells, the following data reflect activity of a mixed population of macrophages and polymorphs. Mean (SD) labelling efficiency with ^{111}In was 46·8 (7)%. With this technique, the dose of indium given to the animals in labelled cells was in the range of 1·85 × 10^6 to 3·7 × 10^6 Bq.

The distribution of radioactivity in tissue samples collected at necropsy is shown in table 4. Activity/g tissue was expressed as multiples of activity in blood. Maximum activity was found in gastric fluid and meconium. Washed small intestine had the highest radioactivity among tissue samples, followed by the stomach and the large intestine. Bone marrow had counts comparable with those in blood. Liver and spleen, however, had counts significantly higher than those in blood and bone marrow ($p<0·05$).

Discussion

The white cells of human colostrum were first observed in 1839 by the biologist and photographer Alexander Donne, and rediscovered in 1966 by Smith and Goldman.[12] Early colostrum contains the highest concentration of cells ranging from 1–3 × 10^{6-8} cells/ml. Toward the end of the first five weeks of lactation the cell concentration drops to about 10^5 cells/ml.[13] Cell counts obtained in our study were within this range.

Table 4 *Radioactivity in specimens obtained from the gastrointestinal tract and systemic tissues. The radioactivity was measured as counts/minute/g sample and expressed as multiples of activity in blood*

Mean (SEM) activity in counts/minute/g sample	
Gastrointestinal lumenal contents:	
Gastric contents	202·0 (64·0)
Meconium	144·0 (37·0)
Washed gastrointestinal tissue:	
Gastric tissue	22·4 (6·0)
Small intestine	142·0 (42·0)
Large intestine	12·4 (5·1)
Systemic tissues:	
Liver	9·5 (4·0)
Spleen	9·1 (2·8)
Bone marrow	1·4 (0·23)
Blood (reference)	1·0

Numerous observations have been made about the functional value of human milk leucocytes, most of which have been based on in vitro studies. Milk cells are actively phagocytic and microbicidal.[8 9] Milk phagocytes adhere to and spread on glass.[8 9] In a recent study human milk macrophages were shown to be motile in collagen gels.[14] Human milk phagocytes elicit antibody dependent cytotoxicity,[15] and give a good metabolic response as measured by chemiluminesence.[16] Whether these in vitro observations hold true in vivo conditions is not known. Our study was designed to investigate the in vivo distribution of milk leucocytes after ingestion by newborn baboons, and three important findings emerged; first, it is possible to use ^{111}In as a marker for human milk leucocytes; secondly, it is likely that some milk leucocytes enter the systemic circulation; and thirdly human milk leucocytes seem to persist in the intestine for a long period after a single feed.

The radioactive tracer ^{111}In labelled to a mixed population of white blood cells has been used in adults for the diagnosis of occult infections.[17–19] The technique of labelling neutrophils with a gamma emitting radionuclide was first evaluated in 1975 by Thakur *et al*[17 18] and has been used in patients since then. The technique is based on the passive diffusion of ^{111}In chelated with 8-hydroxyquinoline. In previous studies investigators have used different markers to study milk leucocytes,[20] but ^{111}In has not been previously reported. In the present study, using relatively small amounts we achieved about 50% labelling efficiency. The technique is comparatively simple and takes less than an hour. The presence of transferrin and fat have been shown to interfere with labelling[17]; this effect can be minimised by thorough washing of the cells.

Awareness that viable leucocytes are present in colostrum and milk has stimulated interest in the importance of these cells.[21] Tests of skin grafting on foster nursed rats and mice have provided circumstantial evidence that, in these species, leucocytes may be transmitted naturally from the mother's blood stream to the suckling's blood stream through the milk.[22] In the present study we have shown that it is possible that some of the milk leucocytes crossed the intestinal barrier of the premature animal and entered its circulation. This speculation stems from analysis of the systemic distribution of ^{111}In at necropsy. One might assume that the high ^{111}In activity found in liver and spleen could represent free isotype. Although we did not have a group of control animals that were given free indium, our interpretation of the distribution data is based on previous studies.[17 19 23 24] ^{111}In does not eluate from the living cells: after death of the cells and release of the ^{111}In it is bound to proteins such as transferrin and is eventually deposited in the bone marrow.[23] Also, because plasma ^{111}In activity tends to remain constant and persists after all labelled granulocytes have disappeared from the blood, it seems that organ activity reflects uptake of cell bound ^{111}In and not plasma ^{111}In.[24] The higher activity in the liver and spleen than in the bone marrow that we found is consistent with the distribution of the radioisotope when labelled leucocytes are injected intravenously.[25]

Such a transfer of milk leucocytes into the systemic circulation after breast feeding in newborn primates has not been reported before, although Beer and Billingham produced strong evidence that it occurred in certain species of rats and mice.[21]

Neither the pH nor the enzyme content of the infants' stomachs are likely to be harmful to the ingested cells[24 26]; in our study the pH of stomach contents was about 7·0. Also the high buffering capacity of milk may be important in protecting its cellular elements.

It has been suggested that breast milk has a protective effect in necrotising enterocolitis and other infectious diseases,[27] but the mechanism of protection is not clear. Kmetz *et al* have shown that when newborn rabbits were fed labelled, viable bovine leucocytes, the cells were found beneath the epithelium of the mucous membranes of the oesophagus and stomach.[28] This apparent ability of mothers to 'transplant' leucocytes naturally to their young through the milk may play an important part in preventing necrotising enterocolitis. In this study we have shown that human milk leucocytes adhere to mucosa or lie intramurally 60 hours after ingestion of a single feed. A recent investigation in which the authors proposed that macrophages may penetrate into mucosal sites for host defense supports this view.[14] If this is true it would make a strong case for the prophylactic administration of human milk leucocytes separàted from the breast milk of lactating mothers to premature infants who otherwise cannot tolerate enteral feeds. There is, however, a risk if the donor is not the infant's biological mother.

A few questions still remain to be answered. We used heterologous milk leucocytes; thus there is difficulty in the interpretation of data on kinetics. The difficulties encountered in conducting such studies in human subjects are well understood, and therefore a non-human primate was used. The second question is whether the radioactivity measured in the gastrointestinal tract and other tissues truly reflects the presence of intact human milk leucocytes or leached ^{111}In. Studies by Thakur *et al* have shown that ^{111}In binds firmly at intracytoplasmic sites, and is usually released only if the cell dies.[17] The released isotope would then be deposited freely in tissues, especially the bone marrow. The fact that a higher amount of radioactivity was found in the liver and spleen and only a minimal amount in bone marrow supports our hypothesis that human milk leucocytes were intact. The mechanism by which human milk leucocytes cross from the gastrointestinal tract into the systemic circulation has yet to be elucidated.

Based on these observations we speculate that human milk leucocytes, once ingested, may remain in the intestine for as long as 60 hours. Those cells that cross the intestine reach sites of the reticuloendothelial system, namely the liver and spleen. The persistence of these cells in the intestinal tract for long periods after a feed suggests the important protective role of milk leucocytes.

References

[1] Goldman AS, Garza C. Future research in human milk. *Pediatr Res* 1987;**22:**493–6.

[2] Ogra SS, Ogra PL. Components of immunologic reactivity in human colostrum and milk. In: Ogra PL, Dayton DH, eds. *Immunology of breast milk*. New York: Raven Press, 1979:185–95.

[3] Rogers HJ, Synge C. Bacteriostatic effect of human milk on Escherichia coli: the role of IgA. *Immunology* 1979;**34:**19–28.

[4] Winberg J, Wessner G. Does breast milk protect against septicaemia in newborn? *Lancet* 1971;**i:**1091–4.

[5] Chandra RK. Prospective studies of the effect of breast feeding on incidence of infection and allergy. *Acta Paediatr Scand* 1979;**19:**694.

[6] Head JR, Beer AE. In vivo and in vitro assessment of the immunologic role of leukocyte cells in milk. In Ogra PL, Dayton DH, eds. *Immunology of breast milk*. New York: Raven Press, 1979:207–25.

[7] Lascelles AK, Gurner BW, Coombs RR. Some properties of human colostral cells. *Aust J Exp Biol Med Sci* 1969;**47:**349–60.

[8] Mohr JA, Leu R, Mabry W. Colostral leukocytes. *J Surg Oncol* 1970;**2:**163–7.

[9] Pitt J. The milk mononuclear phagocyte. *Pediatrics* 1979;**64** (suppl):745–9.

[10] Vidyasagar D, Maeta H, Raju TNK, *et al.* Bovine surfactant (surfactant TA) therapy in immature baboons with hyaline membrane disease. *Pediatrics* 1985;**75:**1132–42.

[11] Ogra SS, Ogra PL. Immunologic aspects of human colostrum and milk II. Characteristics of lymphocyte reactivity and distribution of E-rosette forming cells at different times after the onset of lactation. *J Pediatr* 1978;**92:**550–5.

[12] Smith CW, Goldman AS. The cells of human colostrum I. In vitro studies of morphology and function. *Pediatr Res* 1968;**2:** 103–9.

[13] Xanthou M, Mandyla-Sfagou H, Maravelias C, Baum JD, Matsaniotis N. Phagocytes of human colostrum and their function. In: Stern L, Salle B, Friis-Hansen B, eds. *Intensive care in the newborn III*. New York: Masson, 1981:201–11.

[14] Ozkaragoz H, Rudloff HB, Rajaraman S, Mushtaha AA, Schmalstieg FC, Goldman AS. The motility of human milk macrophages in collagen gels. *Pediatr Res* 1988;**23:**449–52.

[15] Mandyla H, Xanthou M, Maravelias C, Baum D, Matsanistis N. Antibody dependent cytotoxicity of human colostrum phagocytes. *Pediatr Res* 1982;**16:**995–9.

[16] Mandyla H, Xanthou M. Function of leukocytes in human milk. In: Hamosh M, Goldman AS, eds. *Human lactation 2—maternal and environmental factors*. New York: Plenum Press, 1986: 533–40.

[17] Thakur ML, Lavender JP, Arnot RN, *et al.* ^{111}Indium labelled autologous leukocytes in man. *J Nucl Med* 1977;**18:**1014.

[18] Segal AW, Thakur ML, Arnot RN, *et al.* ^{111}Indium labelled leucocytes for localisation of abscesses. *Lancet* 1976;**ii:**1056–8.

[19] Goodwin DA, Doherty PW, McDougall IR. Clinical use of indium ^{111} labelled white cells; an analysis of 312 cases. In: Thakur ML, Goltschalk A, eds. *^{111}Indium labeled neutrophils, platelets and lymphocytes*. New York: Trivirum, 1981:131–45.

[20] Parmerly MJ, Beer AE, Billingham R. In vitro studies on the T lymphocyte population of human milk. *J Exp Med* 1976;**144:** 358–70.

[21] Beer AE, Billingham RE. Immunologic benefits and hazards of milk in maternal-perinatal relationship. *Ann Intern Med* 1975; **83:**865–71.

[22] Goodwin DA, Meares CF. Indium ^{111}II labelled cells. New approaches and radiation dosimetry. In: Thakur ML, ed. *Radiolabelled cellular blood elements*. New York: Plenum Press, 1985:356.

[23] Peters AM, Saverymuttu SH, Lavender JP. Granulocyte kinetics. In: Thakur ML, *Radiolabelled cellular blood elements*. New York: Plenum Press, 1985:356.

[24] Beer AE, Billingham AE, Head J. The immunologic significance of mammary gland. *J Invest Dermatol* 1974;**63:**65–74.

[25] Product information on indium hydroxyquinoline. Amersham: Amersham International, 1986.

[26] Manville IA, Lloyd RW. The hydrogen ion concentration of the gastric juice of fetal and newborn rats. *Am J Physiol* 1932;**100:** 394–401.

[27] Boass A, Wilson JH. Development of mechanisms for intestinal absorption of infamin B^{12} in growing rats. *Am J Physiol* 1963;**204:**101–4.

[28] Kmetz M, Dunne HW, Schultz RD. Leukocytes as carriers in the transmission of bovine leukaemia: invasion of the digestive tract of the newborn by ingested cultured leukocytes. *Am J Vet Res* 1970;**31:**637–41.

[29] Barlon B, Santilli TV, Haird WC, Pitt J, Blanc WA, Schullinger JN. An experimental study of acute neonatal enterocolitis. The importance of breast milk. *J Pediatr Surg* 1974;**5:**587–95.

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Effect of prenatal lignocaine on auditory brain stem evoked response

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SUMMARY To test the hypothesis that there would be a positive correlation between the interpeak wave (I–V) interval as measured by auditory brain stem evoked response and the ratio of umbilical cord blood arterial to venous lignocaine concentrations in infants born after maternal epidural anaesthesia, 10 normal infants born at full term by elective caesarean section were studied. Umbilical cord arterial and venous plasma samples were assayed for lignocaine, and auditory brain stem evoked responses were elicited at 35 and 70 dB at <4 (test 1) and ≥ 48 hours (test 2). Mean wave I–V intervals were prolonged in test 1 when compared with test 2. Linear regression showed the arterial:venous ratio accounted for 66% (left ear) and 43% (right ear) of the variance in test 1 intervals. No association was found in test 2. In newborn infants, changes in serial auditory brain stem evoked response tests occur after maternal lignocaine epidural anaesthesia and these changes correlate with blood lignocaine concentrations.

Lignocaine, one of the most widely used agents in epidural anaesthesia, has been associated with abnormal neurobehavioural responses in the newborn.[1 2] In 1974, Scanlon *et al* reported that lignocaine adversely affected the neonatal behavioural examination (as measured by the Scanlon early neonatal behavioural scale) performed at 2 to 6 hours of life.[3] Subsequent studies failed to confirm these observations. In general, however, these studies lacked both control for confounding variables and appropriate data analyses.[4 5] Several recent studies have confirmed the findings of Scanlon *et al*, showing decreased response to sound and poor state regulation in infants of mothers receiving lignocaine.[6 7] Moreover, experimental studies in animals suggest that lignocaine interferes with neuronal transmission of sound.[8 9] Ruth *et al* found that lignocaine affects the more central portions of the auditory system, especially wave V latency.[9] This was recently confirmed in newborns by Diaz *et al.*[10]

We hypothesised that there would be a positive correlation between the interpeak wave (I–V) interval as measured by auditory brain stem evoked response and the ratio of umbilical cord blood arterial:venous lignocaine concentrations in infants born after maternal epidural anaesthesia. The wave (I–V) interpeak interval rather than the absolute wave V latency was chosen because wave V latency may be affected by external factors—for example, fluid in the ear canal. On the other hand, the wave I–V interval is a direct, uncontaminated measure of neural conduction along the auditory pathways. The ratio of umbilical cord blood arterial:venous lignocaine concentration was chosen because it better reflects actual tissue concentrations.[2]

Patients and methods

We studied 10 normal infants born at full term by elective caesarean section after maternal epidural anaesthesia using lignocaine, 64 mmol/l or 85 mmol/l (molecular weight lignocaine=234·3 g; 1 g lignocaine=42·6 mmol). Only low risk mothers with no complications of pregnancy were enrolled. Infants were tested only if their Apgar scores at one and five minutes were ≥ 7, their growth was appropriate for gestational age, they showed no respiratory distress, and they were normal on physical examination in the observation nursery. Informed consent for cord blood lignocaine measurements and auditory brain stem evoked response testing was obtained from one or both parents before the infant's delivery. The study was approved by the Human Investigation Committee of the University of Michigan Medical Center. Presence or absence of replicated responses at 35 dB nHL (normal hearing level) were determined for each ear. Auditory evoked responses were recorded by surface electrodes attached to the forehead and ipsilateral earlobe or mastoid process with the contralateral ear earthed. Each average consisted of 2000 accepted sweeps. Each averaged response was replicated. The bioelectric activity recorded by the pair of electrodes was amplified differentially (×100 000) and filtered (30–3000 Hz). The auditory brain stem evoked responses were recorded and elicited by means of a Nicolet CA-2000 averager.

Auditory brain stem evoked responses were elicited by broad band clicks presented to each ear at 35 and 70 dB nHL. Each infant was studied at <4 (test 1) and ≥48 (test 2) hours. In addition, interpeak auditory brain stem evoked response (wave I–V) intervals from the 70 dB nHL responses were measured by one observer unaware of the infant's cord blood lignocaine concentration and the study hypothesis. The full protocol was repeated for test 2.

Samples of umbilical cord arterial and venous plasma were assayed for lignocaine using fluorescence immune assay. A segment of umbilical cord was double clamped at delivery, the vessels were identified, and samples were aspirated from each vessel. After centrifugation the plasma was transferred to plastic test tubes and frozen at −48°C until assay could be accomplished. The results were expressed as the ratio of umbilical artery:umbilical venous lignocaine concentrations.

Data were analysed by the Wilcoxon signed rank test; we used a non-parametric statistical test as it could not be assumed that the data were normally distributed (small sample size). The test was two sided. Simple linear regression was also done to study the association between the arterial: venous ratio (predictor variable) and the interpeak (wave I–V) interval measurement (response variable).

Results

Ten mothers and infants were enrolled in and completed the study. The mean (SD) maternal lignocaine dose was 0·023 (0·004) mmol/kg body weight. Mean (SD) birth weight of the study infants was 3157 (421 g) and their mean (SD) gestational age was 39 (1·1) weeks. All were appropriately grown for gestational age. Median Apgar scores at 1 and 5 minutes were 8 (range 7–9) and 9 (all infants), respectively.

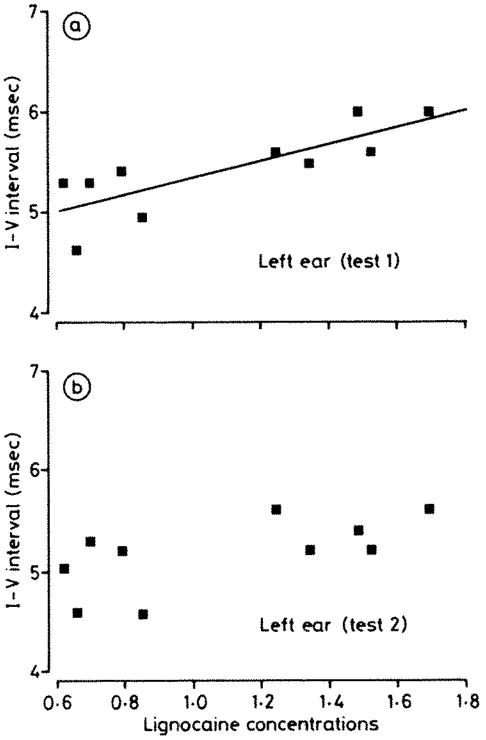

Fig 1(a) *Scattergram showing ratio of arterial: venous cord lignocaine concentrations plotted against wave (I–V) interval as measured by auditory brain stem evoked response at <4 hours postnatal age (left ear). Linear regression y=4·519+0·839 (lignocaine ratio).* (b) *Ratio of arterial: venous cord lignocaine concentrations plotted against wave (I–V) interval as measured by auditory brain stem evoked response at ≥48 hours postnatal age (left ear).*

Mean interpeak (wave I–V) intervals were prolonged in test 1 compared with test 2 for both ears. The difference was greater for the left ear (left: median 5·4 (range 4·6–6·0) compared with 5·2 (range 4·5–5·6) msec (p<0·01) and right: 5·4 (range 4·6–5·8) compared with 5·2 (range 4·6–5·6) (p=0·05)). Two of three infants with arterial:venous lignocaine ratios more than one standard deviation above the mean had no responses for both ears when tested at 35 dB (test 1). Both infants exhibited replicated auditory brain stem evoked responses to 35 dB nHL clicks on test 2.

Mean (SD) plasma lignocaine concentrations were: umbilical artery 3·9 (1·2) mmol/l and umbilical vein 3·8 (1) mmol/l, arterial:venous ratio being 1·1 (0·4). When simple linear regression analyses were performed, the arterial:venous ratio accounted for 65·8% (R^2) of the variance in test 1 wave I–V intervals (r=0·81, p=0·004) as measured in the left ear and 43% (R^2) of the variance, (r=0·66, p<0·04) in test 1 intervals for the right ear. On retest, the regression analyses no longer showed a significant

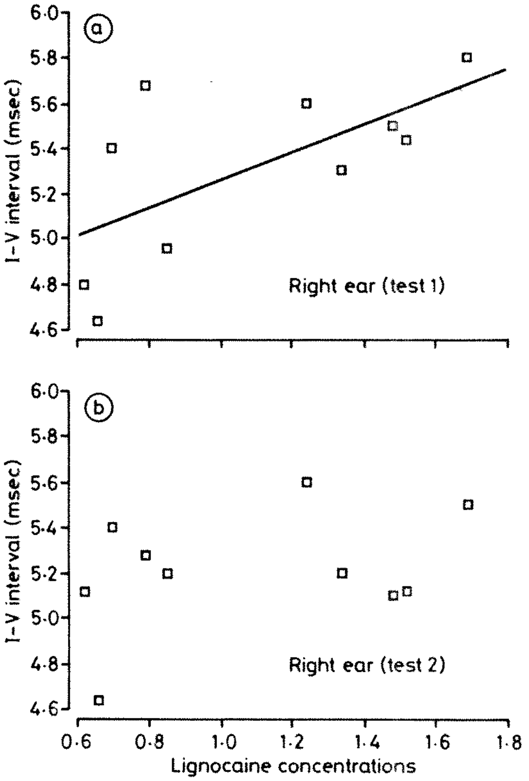

Fig 2(a) *Ratio of arterial: venous cord lignocaine concentrations plotted against wave (I–V) interval as measured by auditory brain stem evoked response at <4 hours postnatal age (right ear). Linear regression y=4·631+0·626 (lignocaine concentration).* (b) *Ratio of arterial: venous cord lignocaine concentrations plotted against wave (I–V) interval as measured by auditory brain stem evoked response at ≥48 hours postnatal age (right ear).*

relationship between test 2 results and the arterial: venous ratio for either ear.

The results are shown in figs 1–3.

Discussion

The results of this study show that there is an association between cord blood lignocaine concentrations after maternal epidural anaesthesia and prolongation of interpeak (wave I–V) intervals as measured on auditory brain stem evoked response. Furthermore, a dose response relationship is suggested by the test 1 results and arterial:venous lignocaine ratios, as well as by results of the regression analyses. The observed differences between the left and right ears were unexpected.

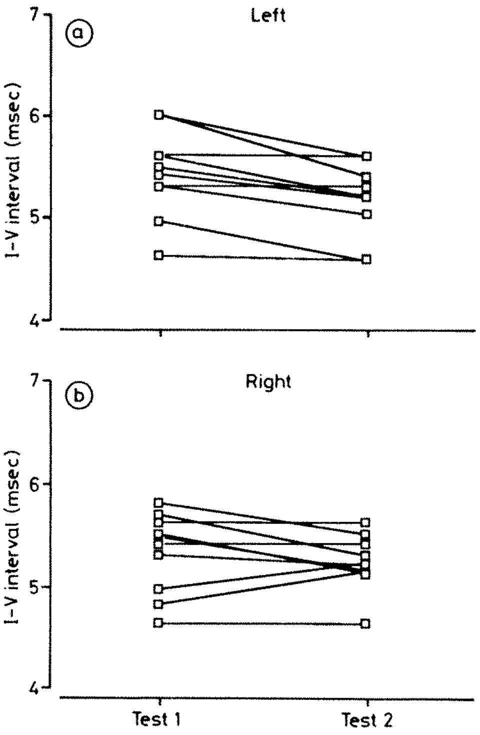

Fig 3(a) *Wave (I–V) intervals for the left ear for each infant at <4 hours (test 1) and ≥48 hours (test 2) postnatal age.* (b) *Wave (I–V) intervals for the right ear for each infant at <4 hours (test 1) and ≥48 hours (test 2) postnatal age.*

Others have found evidence for a increased vulnerability to hypoxic insult in the left hemisphere compared with the right.[11] Asymmetry has also been shown for intracranial haemorrhage, left sided periventricular echo densities,[12] and non-haemorrhagic ventriculomegaly.[14] The difference between the left and right ears in this study may be due to asymmetrical vulnerability.

The controversy over the effect of lignocaine anaesthesia on newborn behaviour began with the report of Scanlon *et al* in 1974.[3] In addition to decreased hearing responses, they found lower test scores on muscular strength and tone. Ostfeld *et al* studied the effect of prenatal lignocaine on hearing responses in the infant born at full term at 4 and 24 hours of age.[7] At both times, they found an abnormal response to sound in 68% of infants, and hypothesised that lignocaine caused a conduction delay in the eighth nerve or other neuronal pathways.

The effect of lignocaine on neural conduction in cats was studied using the auditory brain stem evoked response by Javel *et al*.[8] The auditory brain stem evoked response was recorded after systemic infusion of lignocaine at varying rates. Lignocaine's principal effect on the auditory brain stem evoked response was to lengthen all interpeak intervals without reduction of amplitude. The effects were reversible as the drug concentration fell. They hypothesised that lignocaine produced a uniform, dose dependent shift in central nervous impulse conduction times. They further hypothesised that this shift should have its greatest effect on sensory activities requiring higher central processing, such as speech discrimination. Ruth *et al* recorded the auditory brain stem evoked responses of six healthy male volunteers during intravenous infusion of lignocaine and observed both significant decreases in wave V amplitude and increases in wave V latency.[9]

More recently, Diaz *et al* examined the effect of maternal lignocaine anaesthesia on the auditory brain stem evoked response in 16 infants born at full term.[10] Eight infants were delivered by caesarean section and exposed to lignocaine, while eight were delivered vaginally without maternal anaesthesia. An auditory brain stem evoked response was obtained on all infants at 3–5 hours, and all infants were tested at 30, 60, and 90 dB. A delay in the wave I–V interpeak interval was seen at 90 dB in both ears for the neonates exposed to lignocaine. Surprisingly, no effect was seen at 60 dB. As distortion and cross over to the other ear are more likely at 90 dB, the validity of these findings is not clear. In contrast to our findings, no correlation between lignocaine concentrations and prolongation of wave I–V interpeak intervals was seen. This lack of correlation may be explained by their using absolute umbilical artery or vein lignocaine concentrations as opposed to the arterial:venous ratio that more accurately reflects tissue lignocaine concentration.[2] In our study the mean arterial:venous ratio was greater than 1, indicating that equilibrium between the infants' tissue and blood lignocaine concentrations had been reached. It is possible that the lignocaine arterial: venous ratios were lower in the infants studied by Diaz *et al*. An additional difference from our study was that Diaz *et al* reported no follow up auditory brain stem evoked response results to strengthen the causal hypothesis. Nevertheless, both studies suggest an effect of lignocaine.

Our observational study is limited because of the lack of a control group. The ideal control group would include infants delivered in a similar manner —that is, caesarean section without anaesthesia, which is not possible. All low risk elective caesarean sections are carried out under epidural anaesthesia in our hospital, so we were unable to identify a comparison group also delivered by caesarean section. Even if we were to disregard the possible effect of mode of delivery on auditory brain stem evoked response testing, significant plasma lignocaine concentrations have been found after its use for paracervical block and episiotomy.[15 16] We could not, therefore, identify a sufficient number of deliveries for which no anaesthetic agent was used.

Despite the lack of a control group, our data suggest a causal relationship between epidural lignocaine anaesthesia and alteration in the auditory brain stem evoked response in that (a) there was an association, with a significant dose response gradient, between plasma lignocaine arterial:venous values and interpeak intervals; (b) the cause (lignocaine) preceded the effect; (c) altering the cause (lower plasma lignocaine concentrations) altered the effect (decreased interpeak intervals); and (d) the changes in I–V interpeak intervals from the first few hours to the first few days of life cannot be related to neuromaturation, as maturational changes of this magnitude in interpeak intervals usually take four to six months.

Others have, by behavioural testing, shown an association between maternal lignocaine anaesthesia and several newborn behavioural responses. In that lignocaine has been shown to have a effect on all cerebral neural transmission, our data refine and complement those of Diaz *et al* and support the concept of reported alterations in behavioural responses. As pharmacologically active lignocaine metabolites have been measured in neonatal urine for up to 72 hours,[17] any impact of lignocaine on behaviour or hearing responses in the neonate should be confined to this time period.

References

[1] Avard DM, Nimord CM. Risk and benefits of obstetric epidural analgesia: a review. *Birth* 1985;**12**:215–25.

[2] Kuhnert BR, Linn PL, Kuhnert PM. Obstetric medication and neonatal behavior. Current controversies. *Clin Perinatol* 1985; **12**:423–40.

[3] Scanlon JWS, Brown WU, Weiss JB, Alper MH. Neurobehavioral responses of newborn infants after maternal epidural anesthesia. *Anesthesiology* 1974;**40**:121–8.

[4] Abboud TK, Sarkis F, Blikian A, *et al*. Lack of adverse neonatal neurobehavioral effects of lidocaine. *Anesth Analg* 1983;**62**: 473–5.

[5] Kileff ME, James FM, Dewan DM, Floyd HM. Neonatal neurobehavioral responses after epidural anesthesia for cesarian section using lidocaine and bupivicaine. *Anesth Analg* 1981;**63**: 413–7.

[6] Kuhnert BR, Harrison MJ, Linn PL, Kuhnert PM. Effects of maternal epidural anesthesia on neonatal behavior. *Anesth Analg* 1984;**63**:301–8.

[7] Ostfeld B, Friedman S, Menke T, *et al*. The effect of prenatal lidocaine on hearing responses in term infants. *Pediatr Res* 1985;**19**:356A.

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Plasma amino acid concentrations in parenterally fed preterm infants

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SUMMARY One hundred and nine sick preterm infants were studied, and the data obtained show that hyperphenylalaninaemia is an extremely rare occurrence as long as an adequate source of energy is provided. High concentrations of the other aromatic amino acid (tyrosine) on the other hand, were often encountered and seem to be due to immaturity of an isolated hepatic enzyme as there was no correlation between phenylalanine and tyrosine concentrations. Possible adverse consequences of hypertyrosinaemia are discussed in relation to toxicity and the assessment of hepatic function. We provide reference centiles for plasma amino acid concentrations in this population.

Medical opinion concerning the exact indications for parenteral nutrition in the newborn is far from uniform and its formation is hampered by the lack of prospective randomised trials comparing parenteral with enteral feeding.[1-5] Nevertheless it has been shown that a satisfactory rate of weight gain can be achieved with parenteral nutrition,[5] and it continues to be used regularly in the care of ill newborn babies, especially during intensive care of the very low birthweight baby, and after surgery. Many complications of parenteral nutrition have been documented, some relating to the procedure (such as infection of the infusion line), others concerning the requirements and tolerance of nutrients. Recently concern has been expressed about the amino acid content of one of the most commonly used amino acid solutions, Vamin 9 glucose (KabiVitrum). A series of letters to the *Lancet*[6-9] reported plasma concentrations of phenylalanine in some parenterally fed newborn babies that were well above the upper limit of 600 μmol/l recommended in the management of phenylketonuria.[10] These reports are clearly important in alerting users of Vamin 9 glucose to the possibility of achieving potentially toxic phenylalanine concentrations, and hence of the desirability of monitoring plasma concentrations, but they may not be sufficient justification for changing to other amino acid mixtures in search of lower phenylalanine intakes. Each of the competing products on the market has a unique amino acid profile, and it is quite possible that in removing one apparent problem another might be substituted. Also, of the few controlled trials of parenteral nutrition that have been made, most have used Vamin 9 glucose and so there is relatively less information on the clinical effectiveness of other amino acid solutions in the low birthweight baby.

This study was carried out partly to try to confirm our impression that we had not observed the high plasma phenylalanine concentrations being reported from other centres, and partly to generate reference data for plasma amino acid concentrations in a group of parenterally fed very low birthweight babies. We believed that there was every reason to do the latter because there is relatively little reference data on such babies that has been obtained by quantitative analysis.

Patients and methods

The data were obtained from 109 very low birthweight babies receiving parenteral nutrition as part of intensive care for the problems of prematurity. The mean birth weight was 1277 g (range 488–1454) and the mean gestational age was 29 weeks (range 23–42). Parenteral nutrition was started on the second or third day of life with 0·5 g/kg/day of Intralipid 20% (KabiVitrum), 0·5 g/kg/day of Vamin 9 glucose and 10% dextrose, plus minerals, electrolytes, and vitamins. In the absence of contraindications (such as severe jaundice or sepsis) fat and amino acid intakes were increased daily by 0·5 g/kg/day, to a maximum of 3·0 g/kg/day of amino acid, and 4·0 g/kg/day of fat. Both fat and amino acids were infused continuously throughout each 24 hours. The plasma was screened daily for lipaemia. The plasma triglyceride concentration was monitored weekly on blood taken during the course of the lipid infusion. The concentration was always below 2·0 mmol/l, and usually below 1·5 mmol/l. The intake of 10% dextrose was gradually increased according to the estimated fluid requirements and carbohydrate tolerance, usually up to a maximum of 180 ml/kg/day. At the maximum, the energy intake was about 0·502 MJ/kg/day; an energy:amino acid ratio of 0·167 MJ/g. Plasma amino acid profiles were obtained at weekly intervals so that for most babies studied data are available at different postnatal ages and nutrient intakes. For each amino acid profile 0·3 ml of blood was sampled.

Amino acid analysis was carried out on a Hilger Chromaspeck automated ion exchange amino acid analyser. The biochemistry department participates in the external quality control system (NEQUAS) operated by the Wolfson Research Laboratory, Queen Elizabeth Medical Centre, Birmingham. Statistical analysis was performed using Oxstat (Medstat Ltd).

Results

The table shows the 10th, 50th, and 90th centiles for the plasma concentrations of each amino acid.

To see how plasma amino acid concentrations correlated with gestational age, body weight, and postnatal age, four amino acids were selected—phenylalanine, tyrosine, glycine, and cystine. No significant overall correlations were found in any case, by either parametric (Student's *t*) or by non-parametric (Spearmans rank) tests.

The only significant associations were between the plasma concentration of serine, glutamine, cystine, valine, methionine, isoleucine, leucine, and phenylalanine and the total amino acid intake, such that the concentrations were slightly higher at a dose of 3 g/kg/day than at 1 g/kg/day, and between phenylalanine concentrations and postnatal age, such that the mean concentration rose from 100 μmol/l at 2 days to 187 μmol/l at 10 days (Spearman rank correlation, $p<0·001$).

Fig 1 shows the frequency distribution of plasma phenylalanine concentrations; only four values fell above 500 μmol/l, and only two values above 600 μmol/l. Fig 2 shows the frequency distribution of plasma tyrosine concentrations. Fig 3 shows the association between plasma phenylalanine and tyrosine concentrations sampled simultaneously. It illustrates clearly the lack of correlation between plasma phenylalanine and tyrosine concentrations, which

Table *Plasma amino acid concentrations (μmol/l) by centile (315 observations were made in 109 babies)*

Amino acid	*Centile*		
	10	*50*	*90*
Aspartic acid	32	65	106
Threonine	103	177	306
Serine	183	301	458
Glutamic acid	110	220	376
Glutamine	321	517	780
Glycine	243	358	519
Alanine	186	276	472
Cystine	19	44	75
Valine	129	218	300
Methionine	17	32	51
Isoleucine	30	68	96
Leucine	64	107	150
Tyrosine	75	145	656
Phenylalanine	78	146	266
Histidine	65	95	131
Ornithine	68	122	208
Lysine	84	166	291
Arginine	5	24	58
Proline	181	330	595

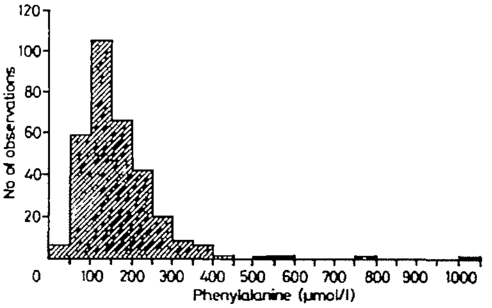

Fig 1 *Frequency distribution of plasma phenylalanine concentrations.*

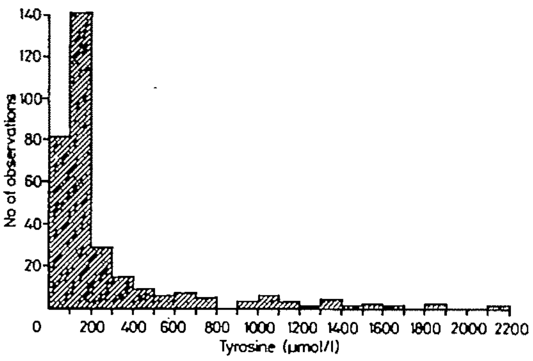

Fig 2 *Frequency distribution of plasma tyrosine concentrations.*

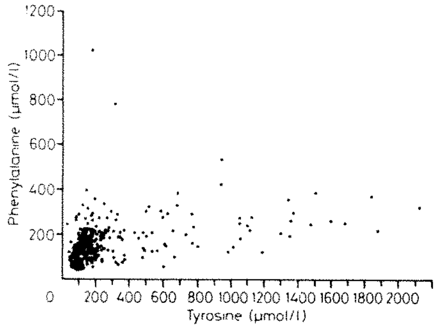

Fig 3 *Association between plasma tyrosine and phenylalanine concentrations sampled simultaneously.*

suggests immaturity of an enzyme specifically concerned with tyrosine metabolism rather than a generalised hepatocellular immaturity.

Discussion

In contrast to several recent reports[6–9] high concentrations of phenylalanine were rarely encountered among this cohort of very low birthweight babies; on only two occasions out of 315 measurements was a plasma phenylalanine concentration greater than 600 μmol/l encountered (fig 1). These high concentrations were both detected in the same baby, who does not have any persisting disorder of amino acid metabolism. Many of the babies whom we studied were undoubtedly ill and were predictably in a catabolic state when their amino acid profile was measured. We have no ready explanation for the variance of our findings with those of other authors, but from our large pool of data, analysed with strict attention to quality control, we are confident in concluding that—when used in combination with an adequate source of non-protein energy—Vamin 9 glucose is a satisfactory source of phenylalanine for the parenterally fed very low birthweight baby.

None of the studies that has reported raised phenylalanine concentrations[6–9] contained information about energy intake, which might be important in explaining the difference between our findings and theirs. Also in some of the studies amino acid values were obtained by thin layer chromatography, which is less accurate than the ion exchange chromatography method that we used. In the light of these studies, however, we would advise that whenever possible total parenteral nutrition should be monitored by the weekly measurement of plasma amino acid concentrations.

Plasma tyrosine concentrations, on the other hand, were often far higher than those regarded as normal in other age groups, including fetal life,[11] and it is well known that preterm babies show limited tolerance to tyrosine because of impaired p-hydroxyphenylpyruvic acid oxidase activity in the immature liver. It is commonly held that high tyrosine concentrations are not harmful, but published reports leave enough room for doubt[12 13] for it to be prudent to respond to high concentrations by reducing amino acid intake. Interestingly, in the formulation of Vamin specifically for infants, namely 'Vamin Infant' (KabiVitrum), there is a reduced phenylalanine content, but the tyrosine content is unchanged.

The poor tolerance of tyrosine exhibited by the very low birthweight infants in this study has implications for the usefulness of the ratio of the plasma concentrations of branched chain amino acids (leucine, isoleucine, and valine) to those of the aromatic amino acids (phenylalanine and tyrosine) as an index of hepatocellular function in this age group. This test is proving to be useful[14 15], and the mechanism is as follows: when hepatocytes begin to lose their ability to generate glucose from glycogen for the use of peripheral tissues, the branched chain amino acids are taken up from the plasma as a substitute energy source. At the same time, the failing hepatocytes are less able to metabolise aromatic amino acids, and so the ratio of branched chain to aromatic amino acids in the plasma decreases. Evidence for the clinical usefulness of this ratio is derived mainly from adult studies, but it has been suggested that it may be relevant in the newborn.[16] We would urge caution in the interpretation of this ratio in the parenterally fed preterm baby because of the high frequency of increased tyrosine concentrations. In 92% of our observations the ratio was less than 2, which is the value said to be characteristic of liver failure in the adult. The increases in plasma tyrosine which we have documented almost certainly result from isolated hepatic enzyme immaturity rather than generalised hepatocellular immaturity, as evidenced by the absence of a significant correlation between plasma concentrations of tyrosine and phenylalanine (fig 3).

The data presented in table 1 provide reference ranges for plasma amino acids in the group that we have described when they were fed Vamin 9 glucose continuously throughout each 24 hours. It can be used as a basis for comparison with other groups and with other nutritional regimens. The absence of any overall correlation between plasma amino acid concentrations and any of the variables that we might have expected to influence them is presumably the result of homoeostatic mechanisms. It has the benefit of allowing the production of reference ranges free of qualifications.

We thank Dr R Jones for his invaluable help with the statistical analysis.

References

[1] Brans YW, Sumners JE, Dweck HS, Cassady G. Feeding the low birthweight infant: orally or parenterally? Preliminary results of a comparative study. *Pediatrics* 1974;**54:**15–22.

[2] Glass EJ, Hume R, Lang MA, Forfar JO. Parenteral nutrition compared with transpyloric feeding. *Arch Dis Child* 1984;**59:** 131–5.

[3] Gunn T, Reaman G, Outerbridge EW, Colle E. Peripheral total parenteral nutrition for premature infants with respiratory distress syndrome: a controlled study. *J Pediatr* 1978;**92**:608–13.

[4] Yu VYH, James B, Hendry P, MacMahon RA. Total parenteral nutrition in very low birthweight infants: a controlled trial. *Arch Dis Child* 1979;**54:**653–61.

[5] Brans YW, Sumners JE, Dweck HS, Bailey PE, Cassady G. Feeding the low birthweight infant: orally or parenterally? Corrected bromide space in parenterally supplemented infants. *Pediatrics* 1976;**58:**809–15.

[6] Bjorkman O, Lindholm M. Phenylalanine content and total parenteral nutrition. *Lancet* 1987;**i:**1311.

[7] Puntis JWL, Edwards MA, Green A, Morgan I, Booth IW, Ball PA. Hyperphenylalaninaemia in parenterally fed newborn babies. *Lancet* 1986;**i:**1105–6.

[8] Walker V, Hall MA, Bulusu S, Allan A. Hyperphenylalaninaemia in parenterally fed newborn babies. *Lancet* 1986;**ii**:1284.

[9] Evans SJ, Wynne-Williams TCJE, Russel CA, Fairbrother A. Hyperphenylalaninaemia in parenterally fed preterm babies. *Lancet* 1986;**ii:**1405–6.

[10] Tourian AY, Sidbury JB. *Phenylketonuria.* In: Stanbury JB, Wyndaarden JB, Fredrickson DA, eds. *The metabolic basis of inherited disease.* New York: McGraw Hill, 1982.

[11] McIntosh N, Rodeck CH, Heath R. Plasma amino acids of the mid-trimester human fetus. *Biol Neonate* 1984;**45:**218–24.

[12] Mamunes P, Prince PE, Thornton NH, Hunt PA, Hitchcock ES. Intellectual deficits after transient tyrosinaemia in the term neonate. *Pediatrics* 1976;**57:**675–80.

[13] Stoerner JW, Butler IJ, Morriss FH, *et al.* CSF neurotransmitter studies: an infant with ascorbic acid-responsive tyrosinaemia. *Am J Dis Child* 1980;**134:**492–4.

[14] Rosen HM, Yoshimura N, Hodgman JM. Plasma amino acid patterns in hepatic encephalopathies of differing aetiology. *Gastroenterology* 1977;**72:**483–7.

[15] Reilly JJ, Halow GM, Gerhardt AL, Ritter PS, Gavaler JS, Van Thiel D. Plasma amino acids in liver transplanation: corrrelation with clinical outcome. *Surgery* 1985;**97:**263–9.

[16] Glasgow JFT, Moore R. Plasma amino acid ratio as an index of hepatocellular maturity in the neonate. *Biol Neonate* 1983;**44:** 146–52.

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Eyelid opening in preterm neonates

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SUMMARY The frequency and patterns of eyelid opening were examined in a group of neonates (gestational age 24–35 weeks). Observations were spread over the 24 hour period to obtain a complete pattern of events. Mean eyelid closure was 74%. When babies were grouped by gestational age, however, differences became apparent, babies of less than 26 weeks having their eyes shut for only 55% of observations compared with 93% at 28 weeks. Trend analysis confirmed that eyelid closure reached a peak at 28 weeks. Neonates exposed to a day/night regimen, opened their eyes significantly more than those exposed to continuous illumination. We suggest that frequency of eyelid opening may be related both to the infant's developmental state (postmenstrual age) and the illumination of the neonatal unit. Frequency of eyelid opening should therefore be considered when calculating the ocular light exposure of preterm neonates.

Patterns of eyelid opening in neonates, particularly if they are born prematurely, are largely unknown. There are only two specific reports on this topic. Berman *et al*[1] estimated that eyelids remained closed for 80% of the time and considered that eyelid closure was a significant factor in limiting the exposure to light of infants at risk of retinopathy of prematurity. Details of the gestational ages of their subjects were not provided. Moseley *et al* investigated the effect of varying illumination on a small group of neonates ranging from 33 to 40 weeks' postmenstrual age,[2] and also observed that eyelids remained closed for about 80% of the time. In this study observations were carried out in a small quiet room immediately after a feed, with observations limited to a three hour study period. To date the pattern of eyelid opening in neonates over a complete 24 hour period has not been reported; this may be relevant to visual development, ocular protection, and growth.

We report an investigation of eyelid opening in preterm neonates. All observations were made in the cots with no interventions of any sort by the observer. This permitted the study of even the most immature preterm neonates. The association between degree of eyelid opening, behavioural state, illumination, and other external events are discussed.

Subjects and methods

Observations were carried out in two neonatal units in the East Midlands: unit A (Leicester Royal Infirmary) and unit B (City Hospital, Nottingham). Both units are divided into high and low dependency nurseries. Artificial lighting is maintained over the 24 hour period in unit A and in the high dependency rooms of unit B. In the low dependency area of unit B, however, artificial illumination is reduced between 2000 and 0700 hours each day. A regimen of reducing night time illumination and noise has been maintained in this area since a previous study[3] attributed improvements in sleep and feeding patterns to these changes. The illumination of the two neonatal units was measured at a series of randomly selected times during a 24 hour period: light intensity in the high dependency nurseries was about 500 lx with no nocturnal decrease. Similarly, the low dependency nurseries had a light level of 500 lx during the day. When the lights had been dimmed in unit B, however, the measured intensity of illumination fell to 1 lx compared with the 500 lx recorded in unit A.

The subjects were 49 preterm infants born between 24 and 35 weeks' gestational age (table 1). All babies of less than 36 weeks' gestation are routinely admitted to the neonatal units studied. Ill babies born at greater than 35 weeks' gestational age are also admitted. Data collected from these older, sick neonates would not have been representative of infants in this age range and therefore observations were restricted to preterm infants. The two groups of babies studied did not differ in the proportion of infants being ventilated (16%) or the proportion being nursed in incubators as opposed to cots (18%).

Table 1 *Details of subjects*

Gestational age (weeks)	*No of infants*	*Birth weight (g)*	
		Median	*(range)*
24	2	0790	0760–0820
25	5	0750	0620–0880
26	7	0855	0650–0980
27	2	1045	1030–1060
28	4	1095	1030–1410
29	11	1370	1020–2120
30	3	1015	0740–1620
31	2	1570	1520–1620
32	5	1750	1220–1859
33	4	1650	1320–2140
34	3	1990	1520–2500
35	1	2320	

SCORING PROCEDURE

Eyelid opening, behavioural state, position of the infant, and amount of handling were recorded. No interventions were made by the observer (JR). The infants were clothed, positioned, fed, and so on, by the mother or nurse and no attempt was made to alter or standardise these variables or to change any baby's schedule in any way.

The scale used to score the degree of eyelid opening was that described by Haith[4] and consisted of a four point scale ranging from 1 (eyelid fully closed) to IV (opening to strain). Scoring of behavioural state was by a four point abbreviated version of the Brazelton scale[5] previously used by Moseley *et al* (table 2).[2] Handling was categorised into: none, feed, nursing procedures, medical interventions, or holding. Position of the infant was recorded as either lying on the left, right, front, back, or being held.

Table 2 *Description of behaviour and state of eyelids*

Behavioural state:	
Category I	Asleep—(Brazelton states 1 and 2) deep or light sleep, little or no motor activity
Category II	Drowsy—(Brazelton state 3): activity variable with mild startles from time to time, movements usually smooth, dazed look when infant is not processing information
Category III	Alert—(Brazelton states 4 and 5): bright look, attention focused, brief fuzzy noises
Category IV	Crying (Brazelton state 6): crying, high degree of motor activity
State of eyelids:	
Category I	Closed—eyelid fully closed
Category II	Chink—partial eyelid opening
Category III	Open—eyelid open, pupil exposed
Category IV	Open to strain—eyelid open, sclera visible above and below the iris

PROCEDURES AND SAMPLING

A maximum of four neonates were studied during each sampling period, which lasted for four hours, because pilot observations had shown that this included a complete cycle between feeds, the most regular disturbance for every neonate.

Each minute during the sampling period, the observer moved round the room and recorded the behavioural state, degree of eyelid opening for each eye, incidence of handling, and position in the cot or isolette for each of the neonates being studied. Care was taken to record handling only if a person was in physical contact with the baby at the moment the observer arrived at the isolette, incubator, or cot. A total of 240 observations (one each minute) was made under each category heading during each four hour sampling period for each baby being observed. If less than four infants were being observed the observer still only allocated 15 seconds to each infant (this included the time taken to travel from cot to cot). Two hundred and eight sampling periods were included in this study (unit A—152; unit B—56). To obtain a complete pattern of events throughout the 24 hour period, the start time of the sampling period was varied throughout the day and night.

The infants were enrolled into the study before the first period of observation, thus it was possible that they could be undergoing phototherapy during the sampling period. In this case the infants were still studied and a note was made if the eyes were covered with phototherapy eyeshields. The data about eyelid opening collected from these infants was not included in the main analysis as an assessment of eyelid state could not be made every minute. After each observation period the health of each infant was graded according to the criteria given in table 3.

ANALYSIS

In each unit eyelid opening was correlated with the occurrence and regularity of interventions and with the infants' behavioural state.

Mean eyelid closure for each age studied (gestational and postmenstrual) was calculated by computing the total number of times that each category of eyelid opening was observed; this value was then divided by the total number of observations made of the state of the eyelids in babies of that age.

Data were then reduced to give one value for each measure for each hour for each infant studied. For example frequency of lid closure was determined by summing the number of observations of eyelid closure (category 1) over each hourly period and then dividing by 60; this value was then converted to a percentage. Data about eyelid state, behavioural

Table 3 *Factors affecting illness score*

	Score
Respiratory support:	
Ventilated	3
Oxygen by headbox or in incubator	2
Breathing air	1
Cardiac support:	
Drugs (for example, dopamine)	1
None	0
Neurological state:	
Paralysed	4
Convulsions, treated	3
Convulsions, untreated	2
Abnormal movements	1
Normal	0
Feeding:	
Gastric feeds	0
Intravenous feeding	1
Phototherapy:	
Yes	1
No	0
Apnoeas:	
No action	1
Requiring stimulation with or without oxygen	2
Bradycardias:	
No action	1
Requiring stimulation with or without oxygen	2

Possible range of scores 0 to 14.

state, and handling were always based on 60 observations each hour. Frequency of occurrence of handling of various types was determined by computing the mean percentage of all observations in which handling was recorded for each hour for each infant.

Results

Mean eyelid closure (category 1) obtained in the first postnatal week (24 to 36 weeks' gestational age) are plotted in fig 1. In general, neonates born between 27 and 31 weeks' gestation had their eyes closed for more than 80% of the total number of the observations. Mean percentage lid closure declined from 80% at 30 weeks' gestation to 60% at 35 weeks' gestation (fig 1). The very immature neonates (24–25 weeks' gestation) opened their eyes for a greater percentage of the time than the older neonates (fig 1), mean percentage eyelid closure at 24 weeks' gestation being 55%. Considerable individual variation was noted in the most immature, however, with values ranging from 60% to 90% for babies born at 26 weeks' gestation. Analysis of trend confirmed that eyelid closure rises to a peak at 28 weeks' gestation (93%). No significant differences were found between eyelid closure as a function of gestational age (observations were made in the first postnatal week) or postmenstrual age (gestational plus postnatal age) (fig 2).

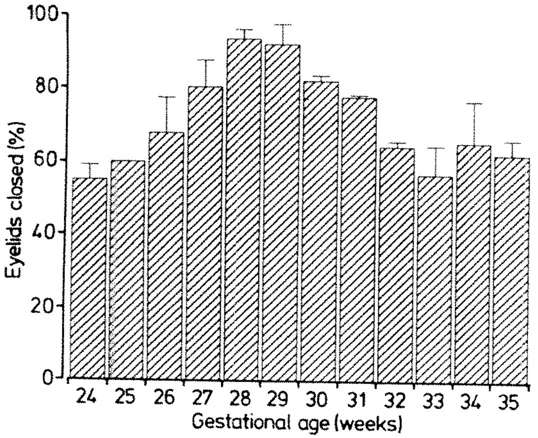

Fig 1 *Percentage eyelid closure and gestational age; observations taken over a 24 hour period during the first week of life are expressed as mean (SEM).*

The pattern and frequency of eyelid opening in the high dependency regions of units A or B were not significantly different. Similarly, during the day, there were no significant differences in eyelid opening in either of the low dependency nurseries. Differences were, however, observed in the fre-

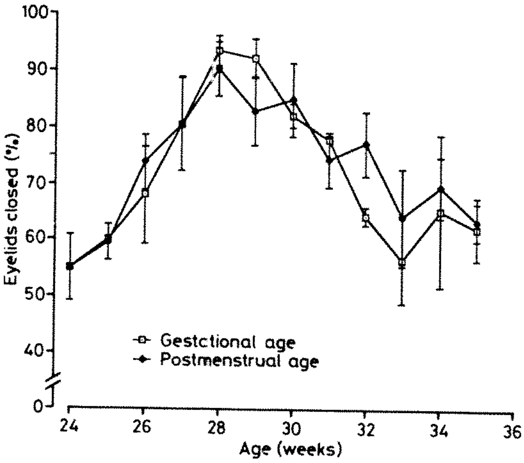

Fig 2 *Percentage eyelid closure, gestational age and postmenstrual age; observations taken over a 24 hour period are expressed as mean (SEM).*

Table 4 *State of eyelids in low dependency regions between 2000 and 0700*

	State of eyelids (%)		
	Shut	*Chink*	*Open*
Unit A	77	5	18
Unit B	64	7	29

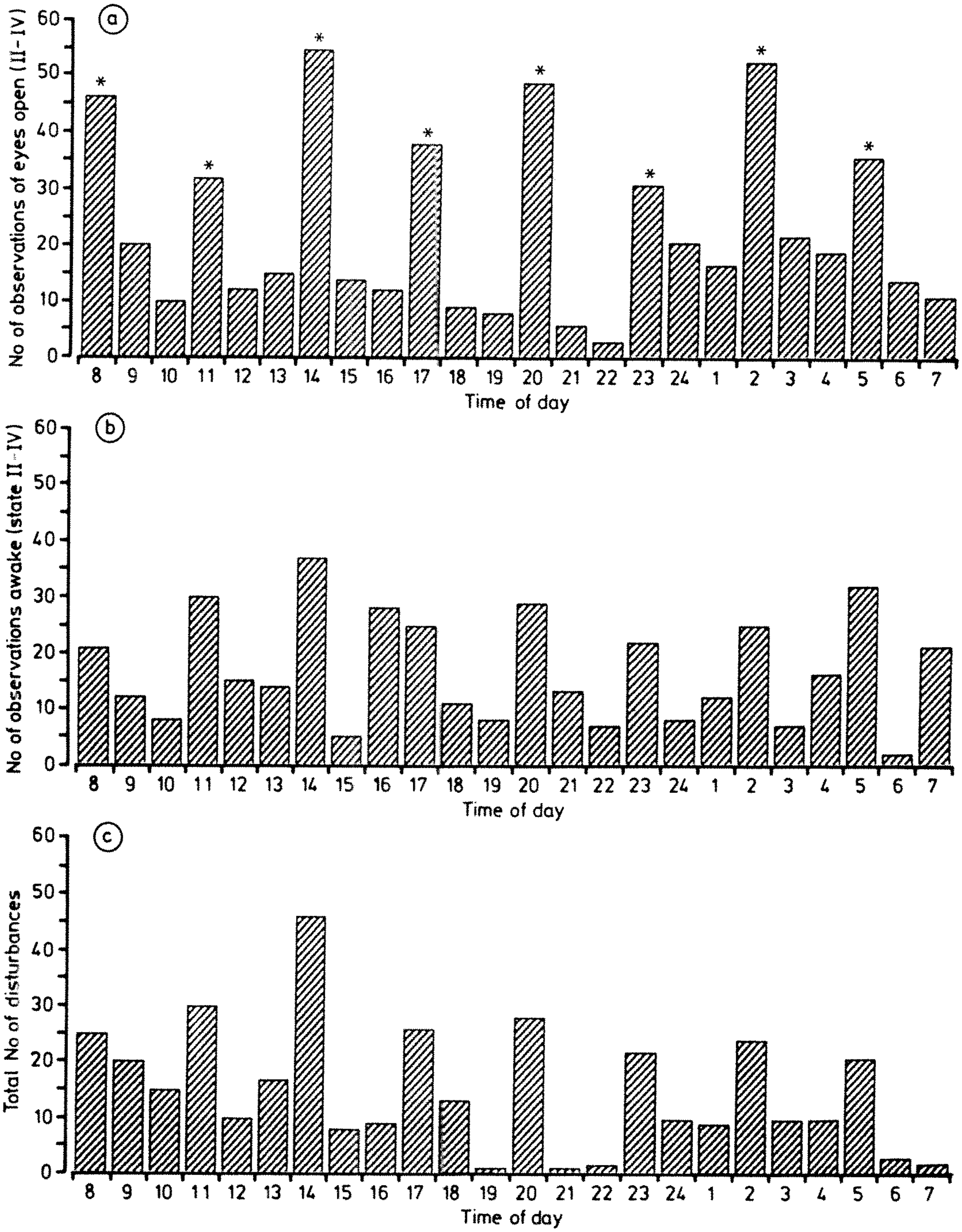

Fig 3 *Histograms of 24 hours for a single infant with a postmenstrual age of 32 weeks in a low dependency nursery showing behavioural state, eyelid closure, and handling. (a) Each bar represents the total number of disturbances each hour; *indicates the times of feeds. (b) Each bar represents the total number of observations of eyelid opening (categories II-IV) each hour. (c) Each bar represents the total number of observations in which the infant was categorised as being awake (states II-IV) in each hour.*

quency of eyelid opening at night between the low dependency nurseries (table 4). Firstly, as the lights were dimmed, babies in unit B responded to the reduction in light with a transient period of eyelid opening lasting about two minutes. Secondly, these infants spent a smaller proportion of the time with their eyes closed (table 4). This difference in mean eyelid closure for unit A (mean 77%, range 47–100%) and unit B (mean 64%, range 23–96%) was significantly ($p<0{\cdot}002$) when compared by multivariate analysis which took the subject's age and health variation into consideration. In addition, the mean duration of periods of eyelid opening intervals (categories II to IV) was similar in units A and B during the day but when the lights in unit B had been dimmed, the average length of time in which the eyes were open increased from 3 to 11 minutes ($p<0{\cdot}001$).

Neonates in unit A were observed to lie with their heads turned to the right for greater than 80% of the observations, while those in unit B spent about half of the time lying on either side.

Analysis of the disturbances to each infant suggested that there was a periodic pattern of events throughout the day with respect to feeds, nursing procedures, and holding, with medical interventions being made as required for the treatment of the babies. A typical summary for a single infant compiled from a series of four hour sampling periods made during the first week of life is shown in fig 3.

During such 24 hour periods infants undergoing intensive care were medically disturbed on average 25 times, while the healthier infants were disturbed less often (10 times) ($p<0{\cdot}005$). No significant differences were found in the number of non-medical interventions between the two high dependency units studied. Both units have a policy of minimal handling—that is, as many disturbances as possible are grouped—even so, these neonates were disturbed more than 100 times each day. Babies in the low dependency region of unit B were held significantly more than the corresponding neonates in unit A during the daylight hours ($p<0{\cdot}002$). No differences were apparent at night.

Neonates within the high dependency areas were asleep (Brazelton states I-II) for 76% of the time. Periods of wakefulness were associated predominantly with either nursing or medical procedures. The maximum number of consecutive observations of 'asleep' was 11—thus the maximum period of uninterrupted sleep was only 11 mintues in either unit, while infants in the low dependency areas had longer periods of uninterrupted sleep (mean 34 minutes).

All infants undergoing phototherapy initially had their eyes covered with protective eye shields. During this study we noted that these shields did not fully cover the babies eyes for 56% of observations (9292 out of 16615) made while neonates were undergoing phototherapy in either of the units studied.

Discussion

During early development the eyelids are fused, usually beginning to open during the sixth month of gestation (24–25 weeks). We found that preterm neonates (24–26 weeks' gestation) had their eyes shut for only 55% of the time, while older neonates opened their eyes less often (fig 1). Little is known about the effects of early exposure to the environment on the developing visual system, this has been reviewed by Fielder *et al.*[6]

The association between gestational age and percentage of time spent with eyelids closed (category I) suggests that neonates of 28–31 weeks' gestation have their eyes shut for more than 80% of the time. This confirms values quoted by Berman *et al* in a study of low birthweight infants of unspecified gestational age.[1] In contrast to Moseley *et al*,[2] who reported that babies of postmenstrual ages ranging from 33 to 40 weeks had their eyes shut for more than 80% of observations, we noted that neonates of 35 weeks' postmenstrual age had their eyes closed for only 55% of observations (fig 1). This apparent difference may be explained by variations in the sampling techniques used in the two studies. Firstly, Moseley *et al* moved the neonates to a small, quiet side room within the neonatal unit for the duration of the study interval immediately after a feed.[2] We noted that the bulk of eyelid opening occurred just before feeds in babies of more than 32 weeks (fig 3), and this would have not been taken into account in the former study.

The changes in the proportion of time spent with eyelids closed (category 1) with increasing postmenstrual age may be attributed to two factors: the development of the lid closure reflex, and the onset of a circadian rhythm. Light causes a reflex closure of the eyelids.[7] Finnstrom has suggested that this reflex may develop around 29–31 weeks' gestation,[8] which corresponds with the maximum percentage eyelid closure found in this study (fig 1). As no significant differences were noted between the pattern of eyelid opening when plotted as either a function of gestational or postmenstrual age (fig 2) this may suggest that exposure to light has not hastened the development of the lid closure reflex in the most immature. If this is the case, the very immature preterm infant (24 to 26 weeks' gestation) may not be able to react to the light stimulus.

The reduction in the amount of eyelid closure after 31 weeks noted in this investigation may reflect the gradual development of a circadian sleep wake rhythm in neonates. Support for this hypothesis is provided by the observation that preterm babies on 'demand' feeding (usually at more than 36 weeks' postmenstrual age) wake and thus open their eyes before feeds. There is evidence that an endogenous circadian rhythm develops spontaneously in the human infant.[9] Booth *et al* have shown that as preterm infants mature they spend a smaller percentage of the time in active sleep, and their quiet sleep periods lengthen.[10] Sleep-wake cycles have been identified in babies from 32 weeks' gestation.[11]

The two neonatal units studied differed in their lighting regimens. Unit B has a policy of reducing the illumination in the low dependency nurseries each evening. We noted that preterm neonates exposed to this 'day/night' regimen opened their eyes more than those subjected to continuous illumination (table 4). This confirms previous observation that neonates often close their eyes when not asleep if in a brightly lit room.[12 13] The observation that neonates also respond immediately to a reduction in illumination with a transient period of eyelid opening confirms a previous study,[2] although these authors were unable to demonstrate the longer term effects that we have identified. Eyelid opening (categories II to IV) for neonates within the low dependency areas, however, occurred particularly in the period before feeds (fig 3)—a period in which observations were not performed by Moseley *et al*,[2] and this may explain the differences between the two studies.

Our results show that the time neonates spend with their eyelids closed is related to gestational (fig 1) or postmenstrual (fig 2) age, and to the level of ambient illumination in the neonatal units in older infants (table 4). The possibility that early exposure to light may be deleterious to the eyes of preterm neonates has recently received much attention.[14] Hamer *et al*,[15] have made an attempt to estimate the retinal irradiance in the eye of an infant of 34 weeks' postmenstrual age undergoing treatment in a neonatal unit. Their calculated value was small relative to those shown to cause retinal damage in animal studies, but did not take into account factors such as eyelid closure and random shading. Our findings suggest that because it occurs so often, estimates of eyelid closure are a prerequisite for calculating the ocular light exposure of neonates.

Quantifying eyelid opening is also relevant to two further topics. First, corneal temperature is lowered by eyelid opening.[16] As most ocular growth occurs between 28 and 40 weeks' gestation,[17] with the cornea in particular flattening during this period, perhaps a thermal deficit may affect ocular development in general and the cornea in particular.[18] Second, visual development, and the early visual experience on this process. The visual experience of these preterm neonates can not be determined without knowledge of the frequency and duration of eyelid opening and clearly this information is a prerequisite to investigating the factors which may influence visual development.

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References

[1] Berman J, Hutchins R, Gold S, Peli E, Lindsey P. Measurement of light exposure in infants at high risk of development retinopathy of prematurity. *Invest Ophthalmol Vis Sci* 1987;**28:** (suppl 3) 119.

[2] Moseley MJ, Thompson JR, Levene MI, Fielder AR. Effects of nursery illumination on frequency of eyelid opening and state in preterm neonates. *Early Hum Dev* 1988;**18:**13–26.

[3] Mann NP, Haddow R, Stokes L, Goodley S, Rutter N. Effect of day and night on preterm infants in a newborn nursery: randomised trial. *Br Med J* 1986;**293:**1265–7.

[4] Haith MH. Visual competance in early infancy. In: Held R, Leibowitz HW, Teuber H, eds. *Handbook of sensory physiology, Vol III: perception*. New York: Springer Verlag; 1978:311–56.

[5] Brazelton TB. Neonatal behavioural assessment scale. *Clinics in developmental medicine. No 88*. 2nd ed. London: Spastics International Medical Publications, Blackwell Scientific, 1984.

[6] Fielder AR, Moseley MJ, Ng Y. The immature visual system and premature birth. *Br Med Bull* 1988;**44:**1093–118.

[7] Peiper A. The development of sensory function: vision. *Cerebral function in infancy and childhood*. Translated for 3rd German ed by Magler B and Magler H. London: Pitman Medical, 1964:52–70.

[8] Finnstrom O. Studies on the maturity in newborn infants III: neurological examination. *Neuropadiatre* 1971;**3:**72–96.

[9] Martin-du-Pan R. La role du rhythme circadian dans l'ailementation du noursisson. *La femme et l'enfant* 1976;**4:**23–30.

[10] Booth CL, Leonard HL, Thoman EB. Sleep states and behaviour patterns in preterm and fullterm infants. *Neuropediatrics* 1980;**11:**354–64.

[11] Parks JD. In: *Sleep and its disorders*. London: WB Saunders, 1985.

[12] Lawson KR, Turkewitz G, Platt M, McCarton C. Infant state in relation to its environmental context. *Infant Behaviour and Development* 1985;**8:**269–81.

[13] Horn MH. Alerting an infant in a brightly lit room. *Critical Care Nurse* 1987;**6:**84–5.

[14] Moseley MJ, Fielder AR. Light toxicity and the neonatal eye. *Clinical Vision Sciences* 1988;**3:**75–82.

[15] Hamer RD, Dobson V, Mayer MJ. Absolute thresholds in human infants exposed to continuous illumination. *Invest Ophthalmol Vis Sci* 1984;**25:**381–8.

[16] Fielder AR, Winder AF, Cooke DE, Bowcock SA. Arcus senilus and corneal temperature in man. In: *The cornea in health and disease*. Royal Society of Medicine International Congress and Symposium Series No 40. London: Royal Society of Medicine, 1981:1015–20.

[17] Weale RA. A biography of the eye: development, growth, age. London: HK Lewis, 1982.

[18] Fielder AR, Levene MI, Russell-Eggitt IM, Weale RA. Temperature—A factor in ocular development. *Dev Med Child Neurol* 1986;**28:**279–84.

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Endogenous formation of prostanoids in neonates with persistent pulmonary hypertension

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SUMMARY Endogenous formation of thromboxane A_2 and prostacyclin were evaluated in seven neonatates with persistent pulmonary hypertension by serial gas chromatographic mass spectrometric determination of their urinary metabolites dinor-thromboxane B_2 and dinor-6-keto-prostaglandin $F_{1\alpha}$, respectively. The patients were studied until their hypertension had resolved on clinical criteria. Urinary excretion of dinor-thromboxane B_2 and dinor-6-keto-prostaglandin $F_{1\alpha}$ was increased when the persistent pulmonary hypertension was associated with group B streptococcal (n=2) and pneumococcal (n=1) sepsis. Based on urinary metabolite excretion, endogenous formation of thromboxane A_2 and prostacyclin did not consistently differ from normal neonates in four patients with non-septic persistent pulmonary hypertension (hyaline membrane disease (n=2), asphyxia, and meconium aspiration). These data suggest that thromboxane A_2 is not a universal mediator of persistent pulmonary hypertension. It may, however, have a role in the pathophysiology of early onset group B streptococcal disease, and persistent pulmonary hypertension of other infectious aetiology. If these findings are confirmed by further studies, thromboxane synthetase inhibition or receptor antagonism may offer a potential therapeutic approach in neonates with persistent pulmonary hypertension associated with sepsis.

There is ample evidence in experimental animals to implicate the vasoconstrictor prostanoid thromboxane A_2 in the pathophysiology of acute pulmonary hypertension.[1–5] These conclusions are based on the effect of pharmacological intervention with cyclo-oxygenase[1 2] and thromboxane synthetase inhibitors,[3] thromboxane receptor antagonists,[5] and the measurements of thromboxane metabolites in plasma,[2 3 5] lung lymph,[1] and urine.[5] Persistent pulmonary hypertension of the neonate is an example of increased pulmonary vascular resistance in the neonatal period and has lead to the hypothesis that thromboxane A_2 might be a modulator of pulmonary vascular tone in this condition. The aim of this study was to assess prostanoid biosynthesis in neonates with persistent pulmonary hypertension and healthy controls using urinary excretion rates of enzymatic metabolites of thromboxane A_2 (dinor-thromboxane B_2) and prostacyclin (dinor-6-keto-prostaglandin $F_{1\alpha}$) as indices of endogenous prostanoid formation.[6]

Patients and methods

Between April and June 1985 at Vanderbilt University Hospital and between March 1985 and January 1988 at the University Children's Hospital, Heidelberg neonates who met the clinical criteria of persistent pulmonary hypertension were enrolled in the study on their first day of life. Patients were excluded from the study if they died early in the course of the disease so that an adequate urine collection could not be made.

Three infants born at full term and four preterm infants (two at Vanderbilt University Hospital, five at Children's Hospital, Heibelberg) were available for study. Clinical suspicion of persistent pulmonary hypertension was based on severe labile hypoxaemia disproportionate to the severity of pulmonary disease and confirmed either by contrast echocardiography, hyperoxic hyperventilation test, or the difference between preductal and postductal arterial oxygen pressure (table).[7] All patients were

Table *Clinical characteristics of patients with persistent pulmonary hypertension*

Gestational age (weeks)	*Birth weight (g)*	*Sex*	*Umbilical artery catheter*	*Central venous line*	*Tolazoline (No of days)*	*$FiO_2>0·21$ (days)*	*Diagnosis*	*Symbol (in figure)*
40	4120	Male	Yes	Yes	10	13	Pneumococcal sepsis	■
38	2700	Female	Yes	No	Not given	10	Group B streptococcal sepsis	◆
33	1650	Male	Yes	Yes	Not given	8	Group B streptococcal sepsis	●
42	2870	Male	Yes	No	3	9	Perinatal asphyxia	□
40	4110	Female	Yes	Yes	3	17	Meconium aspiration	△
31	1600	Female	Yes	Yes	Not given	19	Hyaline membrane disease	◇
31	1470	Female	Yes	Yes	1	16	Hyaline membrane disease	○

mechanically ventilated with a maximum fractional inspiratory oxygen (FiO_2) value of 1·0 on day 1. FiO_2 requirements could be decreased to room air at a median of 13 (range 8–19) days. Mechanical ventilation was necessary for a median of 10 (range 5–19) days. Four of the infants were treated with tolazoline. One patient with meconium aspiration had an episode of thrombocytopenia, the lowest platelet count being $42\times10^9/l$ on day seven. None of the babies died.

Urine was collected for 12 to 24 hours on two to seven occasions during the first 12 days of life until the persistent pulmonary hypertension had clinically resolved. Collection was made in self adhesive bags or from an indwelling catheter, if clinically indicated, and urine volume was determined. Analysis of dinor-thromboxane B_2 and dinor-6-keto-prostaglandin $F_{1\alpha}$ by gas chromatography negative ion chemical ionisation mass spectrometry was carried out as described previously.[8] Prostanoid metabolite excretion was expressed as ng/h/1·73 m^2. The normal range of prostanoid metabolite excretion during the neonatal period was determined on 56 occasions in 29 neonates some born preterm and some at full term, using the same methods.

The study protocol was approved by the ethics committees of both hospitals.

Results

Excretion of the thromboxane metabolite dinor-thromboxane B_2 varied considerably among the individual patients (fig 1). Whereas the neonates with persistent pulmonary hypertension associated with sepsis had excretion rates consistently above the normal range early in their course, the three other patients without sepsis (cases 4, 6, and 7 in the table) did not exceed control values. Case 5 (with severe perinatal asphyxia and meconium aspiration) had normal dinor-thromboxane B_2 excretion at the peak of the disease, which increased as the thrombocytopenia developed, and the FiO_2 went down to 0·5. Metabolite excretion reflected the clinical course—for example, the fraction of inspired oxygen as a global index of right to left shunting in cases 1 and 2. Case 3, however, was unique in that his dinor-thromboxane B_2 excretion peaked at a time when the pulmonary hypertension had resolved clinically and oxygen requirements had decreased to room air. For this reason, no further follow up data are available.

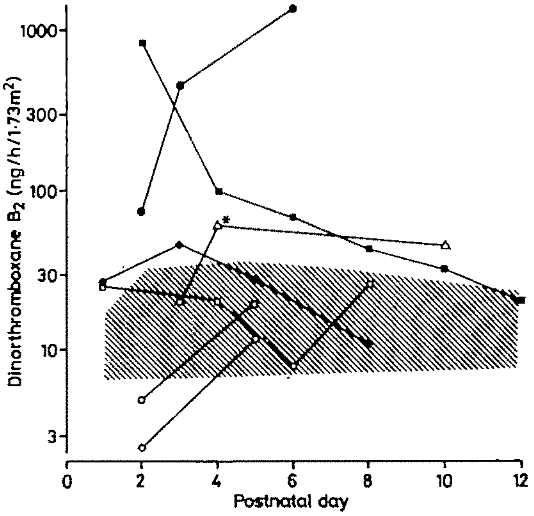

Fig 1 *Urinary excretion of dinor-thromboxane B_2 (logarithmic scale). Symbols for each patient are given in the table. Shaded area=normal range. *Episode of thrombocytopenia.*

The association between FiO_2 requirements and the excretion rates of dinor-thromboxane B_2 is shown in fig 2. There was no positive correlation

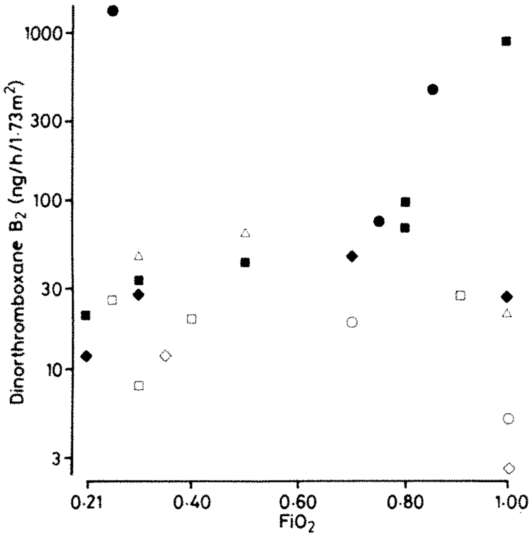

Fig 2 *Relationship between FiO_2 and excretion rates of dinor-thromboxane B_2 (logarithmic scale). Symbols for each patient are given in the table.*

between these two variables in patients with persistent pulmonary hypertension not related to sepsis ($r=-0.39$ by linear regression analysis). In neonates in whom it was associated with sepsis, however, the two variables showed a trend towards positive correlation: linear regression analysis $r=0.33$ ($0.05<p<0.1$).

Excretion rates of the prostacyclin metabolite dinor-6-keto-prostaglandin $F_{1\alpha}$ followed the general pattern of the thromboxane metabolite (fig 3).

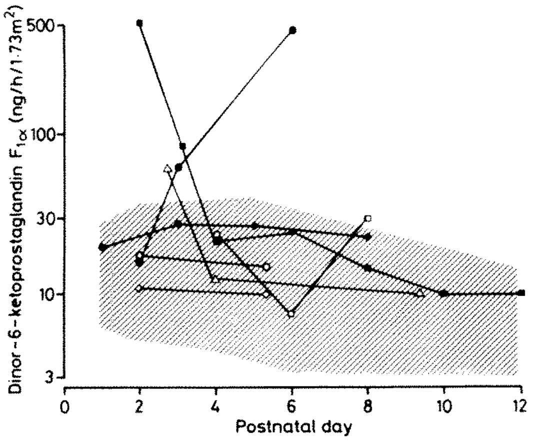

Fig 3 *Urinary excretion of dinor-6-keto-prostaglandin $F_{1\alpha}$ (logarithmic scale). Symbols for each patient identification are given in the table. Shaded area=normal range.*

Again, patients with pulmonary hypertension associated with sepsis excreted more dinor-6-keto-prostaglandin $F_{1\alpha}$ than their non-septic counterparts.

Mean (SEM) urine output during the sampling periods was comparable in the two groups: 3.3 (0.3) ml/kg/h for patients with hypertension associated with sepsis and 3.2 (0.7) ml/kg/h for those without sepsis. Thus differences in urine output do not account for variations in metabolite excretion.

Discussion

The present study showed that urinary metabolite excretion was consistently higher early in the course of pulmonary hypertension associated with sepsis compared with hypertension without sepsis. The two groups of patients resembled each other in many respects—for example, the degree of ventilator dependency, oxygen requirements, and the number of central venous and arterial lines. The latter factor has been shown to increase excretion of both the thromboxane and prostacyclin metabolites.[5 9] The pattern of thromboxane and prostacyclin metabolite excretion, both in neonates with persistent pulmonary hypertension and normal neonates, agrees well with the results previously found in extremely low birthweight infants (less than 1000 g), although more immature and probably sicker premature infants tend to have higher excretion rates.[8] Serial determinations of metabolite excretion did not mirror the clinical courses in non-septic patients with persistent pulmonary hypertension, whereas an association with FiO_2 requirements in pulmonary hypertension associated with sepsis is suggested. In the only patient with thrombocytopenia, thromboxane metabolite excretion reflected the decreasing platelet count rather than the course of pulmonary vasoconstriction, as shown by the oxygen dependency. Thus other factors—for example, platelet activation—have to be considered in the interpretation of increased thromboxane biosynthesis. Our results suggest that in persistent pulmonary hypertension, thromboxane A_2 is not apparently the only modulator of increased pulmonary vascular tone and that the relevance of thromboxane A_2 depends on the aetiology of the pulmonary hypertension. These data support the hypothesis that thromboxane A_2 may contribute to the pathophysiology of sepsis, as suggested by animal experiments.[1-5]

On the other hand, these results of urinary thromboxane metabolite excretion are at variance with increased immunoreactive plasma thromboxane B_2 reported in persistent pulmonary hypertension or predominantly non-infectious aetiology by Hammerman *et al*[10] and Ford *et al*.[11] Circulating plasma concentrations of thromboxane B_2 have been estimated in the 1–2 pg/ml range in adults by infusion studies.[12] Although plasma concentrations may be higher in neonates,[8] plasma concentrations in the 30–3000 pg/ml[10] or even 10–60 ng/ml[11] range most likely reflect in vivo platelet activation rather than genuine circulating thromboxane B_2 as an index of endogenous thromboxane A_2 formation.[6 13 14] Likewise the differerence between normal controls and those with persistent pulmonary hypertension[10] may mirror the fact that platelets in the latter may be more 'sticky' and easily activated, as shown by the incidence of thrombocytopenia in this study. The course of thromboxane metabolite excretion in our only thrombocytopenic patient is in agreement with this assumption. In the more standardised setting of an animal experiment, Hammerman *et al*[15] were able to distinguish between pulmonary hypertension associated with sepsis and that associated with hypoxia on the basis of plasma thromboxane concentrations, which supports these data.

Any evaluation of endogenous thromboxane A_2 and prostacyclin formation in clinical studies should rely on a dependable analytical approach.[16] In this respect, thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$ in plasma are indices that may readily be confounded by in vivo artefacts.[6 14 16] Urinary enzymatic metabolites have been shown to be useful analytical targets in various clinical studies.[5 14 16] Our data obtained by such an approach show that endogenous thromboxane and prostacyclin formation are increased early in the course of persistent pulmonary hypertension associated with sepsis. If experience with more patients should confirm these results, new treatment modalities by thromboxane synthase inhibitors or receptor antagonists might improve the prognosis of certain subtypes of persistent pulmonary hypertension. In view of the increase in endogenous prostacyclin formation, however, exogenous administration of this potent pulmonary vasodilator does not seem to be a logical therapeutic intervention in persistent pulmonary hypertension associated with sepsis.

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References

[1] Rojas J, Larsson LE, Ogletree ML, *et al.* Effects of cyclooxygenase inhibition on the response to group B streptococcal toxin in sheep. *Pediatr Res* 1983;**17**:107–10.

[2] Runkle B, Goldberg RN, Streitfeld MM, *et al.* Cardiovascular changes in group B streptococcal sepsis in the piglet: response to indomethacin and relationship to prostacyclin and thromboxane A_2. *Pediatr Res* 1984;**18**:874–8.

[3] Truog WE, Sorensen GK, Standaert TA, Redding GJ. Effects of the thromboxane synthetase inhibitor, dazmegrel (UK 38,485), on pulmonary gas exchange and hemodynamics in neonatal sepsis. *Pediatr Res* 1986;**20**:481–6.

[4] Brigham KL, Meyrick B. State of art: endotoxin and lung injury. *Am Rev Respir Dis* 1986;**133**:913–27.

[5] Kühl PG, Bolds JM, Loyd JE, *et al.* Thromboxane receptor mediated bronchial and hemodynamic responses in ovine endotoxemia. *Am J Physiol* 1988;**254**:R310–9.

[6] FitzGerald GA, Pedersen AK, Patrono C. Analysis of prostacyclin and thromboxane biosynthesis in cardiovascular disease. *Circulation* 1983;**67**:1174–7.

[7] Fox WW, Duara S. Persistent pulmonary hypertension in the neonate: diagnosis and management. *J Pediatr* 1983;**103**;505–14.

[8] Kühl PG, Cotton RB, FitzGerald GA. Systemic production of prostacyclin and thromboxane A_2 does not correlate with patency of the ductus arteriosus in very low birth weight infants. *J Pediatr* 1986;**108**:977–82.

[9] Roy L, Knapp HR, Robertson RM, FitzGerald GA. Endogenous biosynthesis of prostacyclin during cardiac catheterization and angiography in man. *Circulation* 1985;**71**:434–40.

[10] Hammerman C, Lass N, Strates E, *et al.* Prostanoids in neonates with persistent pulmonary hypertension. *J Pediatr* 1987;**110**:470–2.

[11] Ford WDA, James MJ, Walsh JA. Congenital diaphragmatic hernia: association between pulmonary vascular resistance and plasma thromboxane concentrations. *Arch Dis Child* 1984;**59**:143–6.

[12] Patrono C, Ciabattoni G, Pugliese F, *et al.* Estimated rate of thromboxane secretion into the circulation of normal man. *J Clin Invest* 1986;**77**:590–4.

[13] Schweer H, Kammer J, Kühl PG, Seyberth HW. Determination of peripheral plasma prostanoid concentration: an unreliable index of 'in vivo' prostanoid activity. *Eur J Clin Pharmacol* 1986;**31**:303–5.

[14] FitzGerald GA, Healy C, Daugherty J. Thromboxane A_2 biosynthesis in human disease. *Fed Proc* 1987;**46**:154–8.

[15] Hammerman C, Komar K, Abu-Khudair H. Hypoxic vs septic pulmonary hypertension. *Am J Dis Child* 1988;**142**:319–25.

[16] Seyberth HW, Kühl PG. The role of eicosanoids in paediatrics. *Eur J Pediatr* 1988;**147**:341–439.

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Regular review

Magnetic resonance and near infrared spectroscopy for investigation of perinatal hypoxic-ischaemic brain injury

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The introduction of non-invasive methods for the assessment of cerebral structure and function has been one of the most exciting recent developments in neonatal paediatrics. The availability of these methods permits the incidence and mechanisms of potentially brain damaging influences to be explored, preventive measures and treatments to be tested, and the prognosis of small sick infants to be determined with reasonable confidence. The ability to study these problems has important implications for the conduct of neonatal intensive care.

Ultrasound imaging has proved to be a particularly useful method that has provided much information about cerebral (periventricular) haemorrhage in preterm infants. It has also confirmed the serious prognostic significance of the most florid lesions of periventricular leucomalacia, which results from hypoxic-ischaemic brain injury. Follow up studies of cohorts of preterm infants prospectively studied with ultrasound imaging leave us in little doubt that hypoxic-ischaemic injury is a more important cause of permanent neurodevelopmental disability in surviving very preterm infants than periventricular haemorrhage. Birth asphyxia in infants born at full term also causes hypoxic-ischaemic injury to the brain and accounts for more disabled survivors in the community than result from very preterm birth.[1] It seems clear that hypoxic-ischaemic injury is the commonest cause of perinatally acquired damage to the brain.

The purpose of this review is to describe two relatively new methods for the non-invasive investigation of this type of brain injury: magnetic resonance spectroscopy and near infrared spectroscopy. They provide complementary information about cerebral oxygenation and haemodynamics and are likely to become widely used for clinical as well as research purposes.

Magnetic resonance spectroscopy

The first observations of the human brain by magnetic resonance spectroscopy were made in the early 1980s from newborn infants.[2] [3] For observations to be made, the baby has to be transported to the spectrometer and inserted within the bore of a superconducting magnet. The studies are conducted well within National Radiological Protection Board guidelines and the technique seems completely safe, provided that care is taken to exclude ferromagnetic metal from the vicinity of the magnet. The infants can be monitored and if necessary mechanically ventilated during the studies, but ethical constraints preclude the transport of unstable babies to the magnet for research purposes.

Magnetic resonance spectroscopy can be used to measure the concentrations in brain tissue of phos-

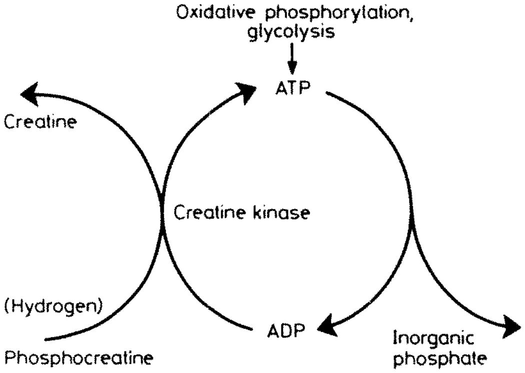

Fig 1 *'High energy' phosphorus energetics. ATP, phosphocreatine (PCr) and inorganic phosphate (Pi) can be measured by magnetic resonance spectroscopy.*

phorus metabolites that are important in energy metabolism, notably adenosine triphosphate [ATP], phosphocreatine [PCr] and inorganic orthophosphate [Pi].[4] Intracellular pH can also be measured. In situations where oxidative phosphorylation is impaired, either because the oxygen supply to the brain is reduced, or the mechanisms for consuming oxygen are damaged, [ATP] will fall (fig 1); the fall is initially extremely small, however, because of the buffering effect of the creatine kinase reaction. This reaction maintains [ATP] close to normal, but [PCr] falls and [Pi] increases—in other words the [PCr]/[Pi] ratio decreases. This ratio is proportional to the phosphorylation potential ([ATP]/[ADP][Pi]) of the tissue and is commonly used as a measure of its energy reserve. [ATP] becomes appreciably depleted only when the [PCr] has fallen to a very low level.

The principle of the technique depends on the fact that the nuclei of phosphorus and certain other atoms, for example hydrogen, ^{13}carbon, sodium, and fluorine have a magnetic moment. They tend to align themselves along the strong magnetic field. If a radio frequency pulse at the appropriate wavelength is applied at right angles to the field by a coil on the surface of the baby's head, this alignment is disturbed. When the pulse ceases, the nuclei return to their previous alignment and generate a magnetic resonance signal, which can be detected. The frequency or frequencies of the signal defines the compounds present, and the signal intensity is proportional to concentration.

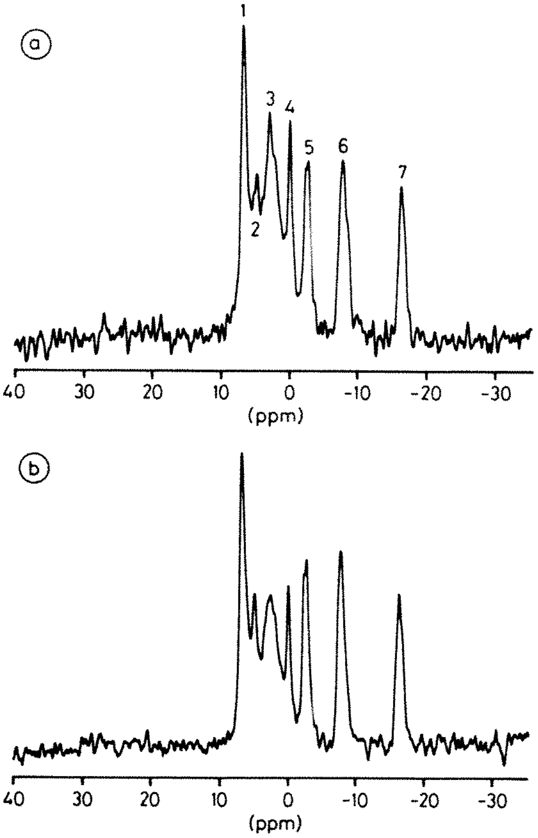

Fig 2 *Phosphorus spectra from two infants with normal brains: (a) born at term and (b) born at 28 weeks' gestation; both were studied during the first week of life. The x axis is chemical shift (ppm) relative to the resonance frequency of PCr, and the y axis is signal intensity. The peaks (1–7) are attributable to phosphomonoesters, Pi, phosphodiesters, PCr, and the γ, α, and β phosphorus nuclei of ATP, respectively. (Data of Azzopardi et al.[5])*

THE NORMAL BRAIN

Fig 2 shows phosphorus spectra from the temporoparietal cortex of two infants with normal brains, one (a) born three months preterm, and the other (b) born at full term. The seven numbered spectral peaks are attributable, from left to right, to (1) phosphomonoesters, (2) Pi, (3) phosphodiesters, (4) PCr, and (5), (6), and (7), the γ, α, and β phosphorus nuclei of nucleotide triphosphates, mainly ATP. The x axis is a frequency axis and the y axis is signal intensity: the areas under the peaks are proportional to concentration. The PCr peak is proportionately larger and the Pi peak smaller in the full term than in the preterm infant. Studies of a group of 30 infants with normal brains have shown that [PCr]/[Pi] increased significantly with maturation of the brain from 27 to 42 weeks' gestational plus postnatal age, implying an increase in phosphorylation potential.[5] At the same time the relative concentration of phosphomonoesters (mainly phosphoethanolamine) fell and that of phosphodiesters (including phosphatidylcholine and phosphatidylethanolamine) rose. These changes are related to the increasing complexity of membranes and to the formation of myelin. Data from infants who were small for gestational age but otherwise healthy seemed no different from those from appropriately grown infants. Cerebral intracellular pH (measured by a version of the Henderson-Hasselbach equation from the difference in resonance frequency between PCr and Pi[6]) appeared not to change with gestation and was about 7·10 to 7·15.

Phosphorus spectra from the brains of newborn animals show changes similar to those found in the human infant. By comparison, the infant seems to be at about the same stage of maturation, in terms of phosphorus metabolites, as other altricious animals such as the rat, but less mature than the lamb or guinea pig, which are precocious.[7 8]

HYPOXIC-ISCHAEMIC BRAIN INJURY

Birth asphyxia

Birth asphyxia is the form of injury most thoroughly studied by magnetic resonance spectroscopy so far.[2 9–13] The acute effects of severe interruption of oxygen supply to the brain of the human infant during labour have not been investigated for practical reasons, but some inferences about the likely changes may be drawn from the results of studies of laboratory animals.[14–17]

Fig 3 shows spectra from a newborn lamb whose cerebral oxygen supply was acutely curtailed by clamping the carotid arteries and reducing the arterial oxygen tension (PaO_2).[14] During the hypoxic-ischaemic episode [PCr] fell sharply, [Pi] rose greatly, and [ATP] became depleted. At the same time the intracellular pH fell to 6·38 as a result of the production of lactic acid, with the intracerebral lactate concentration, measured by ^1H (proton) magnetic resonance spectroscopy, increasing to 11 mmol·kg^{-1}. When brain oxygenation was restored, considerable recovery had occurred by two hours. Similar results have been found in various animal species and confirm that recovery of the phosphorus metabolites and intracellular pH after a severe hypoxic-ischaemic episode can be rapid—and often complete within an hour or so[14 15]—though lactate may sometimes persist in brain tissue after the intracellular pH has returned to normal.[14]

The effects of intravenous infusion of glucose solution during, and sodium bicarbonate after, a severe ischaemic episode have been tested in lambs.[15] In newborn rabbits the results of bicarbonate infusion after moderate hypoxaemia have also been observed.[16] The main finding was that in none of the studies were important adverse effects in reducing intracellular pH detected—a conclusion that seems at variance with previous investigations using other methods in adult animals.[18] In another study the effects of alterations in arterial carbon dioxide tension ($PaCO_2$) on intracellular pH were observed in lambs: homoeostasis seemed rather greater than expected.[8]

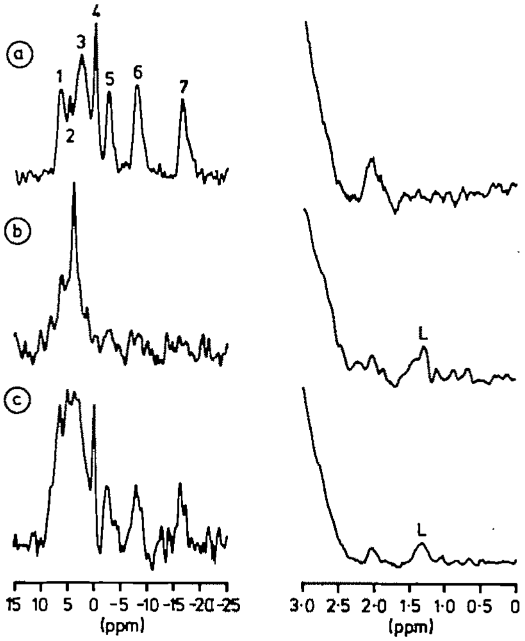

Fig 3 *Phosphorus (left) and proton (^1H) (right) spectra from the brain of a newborn lamb. The peak assignments (1–7) are attributable to phosphomonoesters, Pi, phosphodiesters, PCr, and the γ, α, and β phosphorus nuclei of ATP respectively. L=lactate; (a)=control spectra, intracellular pH 6·97; (b)=during severe acute hypoxia-ischaemia, intracellular pH 6·38; (c)=two hours after hypoxia-ischaemia, intracellular pH 7·23. (Data of Hope et al.[14])*

Studies of newborn infants who have sustained severe birth asphyxia show, perhaps surprisingly, that the phosphorus spectra and intracellular pH were often normal on the first day of life, but abnormalities developed during the next few days in spite of satisfactory values for PaO_2, $PaCO_2$, pH, and blood glucose concentrations.[9–13] The abnormalities initially seemed similar to those encountered during an acute hypoxic-ischaemic episode in laboratory animals and illustrated in fig 3, although they developed much more slowly. The first changes were a fall in [PCr] and a roughly reciprocal rise in [Pi], followed by a fall in [ATP] (expressed as a fraction of total phosphorus signal). These changes indicated severe impairment of oxidative phosphorylation (fig 1). Unlike the situation in acute hypoxia-ischaemia, however, intracellular pH tended to rise. Later [Pi] increased further, well out of proportion to the fall in [PCr], and in fatally damaged brains PCr and ATP virtually disappeared.[12 19] In less severely affected infants who recovered, the phosphorus metabolite ratios returned to normal over the course of about two weeks, but sometimes the total phosphorus signal appeared reduced, indicating permanent loss of brain cells.[9 11] Typical spectra from a baby with birth asphyxia are shown in fig 4.

The origin of the above sequence of events is likely to lie in an acute intrapartum hypoxic-ischaemic episode, with effects similar to those illustrated in fig 3. It can be surmised that an episode of this kind, which may be termed 'primary energy failure', initiates a series of reactions which later cause slowly progressive disruption of oxidative phosphorylation in brain tissue. This disruption may be designated 'secondary energy failure'. Damage to the mitochondrial respiratory electron transport chain as a result of the toxic effects of excitatory neurotransmitters, calcium influx, and free radicals may all play a part, causing failure of utilisation of available oxygen.[18 20 21] Inadequacy of oxygen supply associated with cerebral oedema and other factors is also likely to be concerned. The rise, rather than fall in intracellular pH, which seems to distinguish secondary from primary energy failure, may be attributed to severe disruption of membrane transport mechanisms.

It has been suggested by Chance *et al* that it may be possible to interpret the observed sequence of changes in the brain in terms of metabolic heterogeneity of cells, and that the proportion of seriously compromised and dead cells may be estimated from the magnetic resonance spectroscopy data.[22]

The finding that the phosphorus spectra were usually normal soon after delivery suggests the possibility of early therapeutic intervention to prevent secondary energy failure. Much work is currently under way to explore the effects of 'cerebroprotective' agents such as excitatory neurotransmitter antagonists, calcium entry blockers, and free radical scavengers. The results of investigations

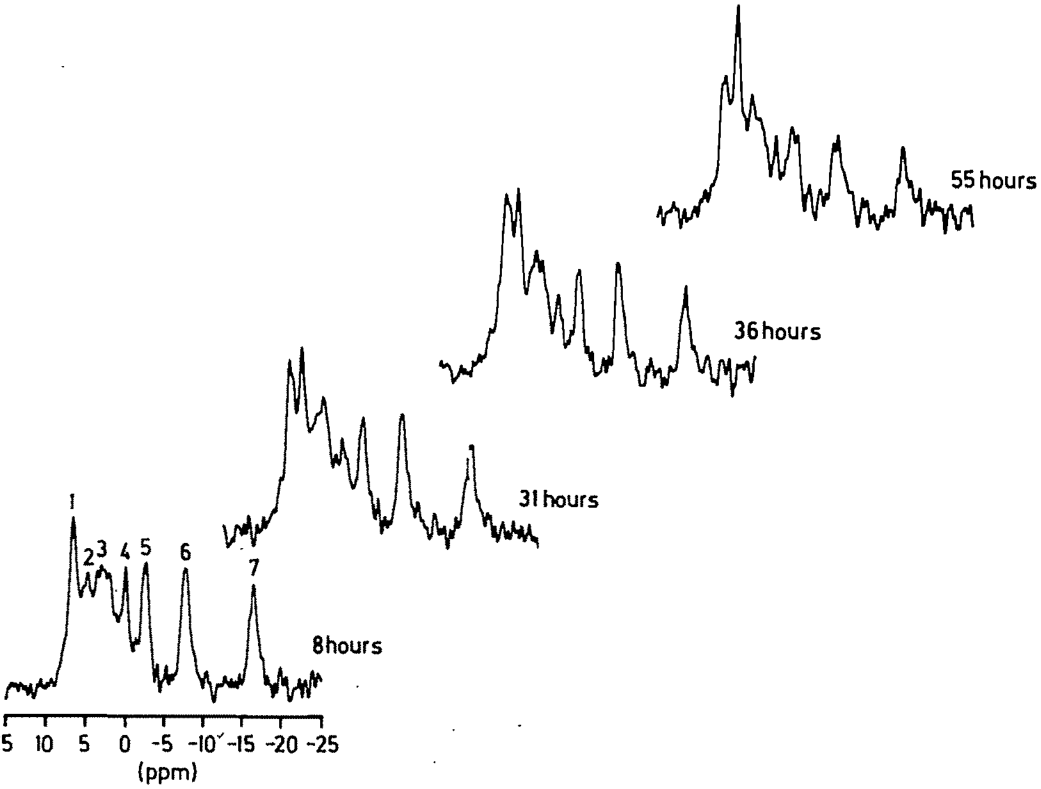

Fig 4 *Phosphorus spectra from a baby born at 37 weeks' gestation who had sustained severe birth asphyxia. The peaks (1–7) are attributable to phosphomonoesters, Pi, phosphodiesters, PCr, and the γ, α, and β phosphorus nuclei of ATP, respectively, and the age at the time of each study is indicated. At 8 hours the spectrum was normal, but [PCr]/[Pi] fell to a minimum value of 0·32 at 55 hours, when [ATP]/[total phosphorus] also fell, to 0·04. Intracellular pH rose to a maximum of 7·28 at 36 hours. The infant died aged 60 hours. (From Azzopardi et al.[13])*

in laboratory animals are encouraging, and suggest that the early administration of these agents may limit or prevent death of brain cells.[21 23-25] While there is no doubt that avoidance of birth asphyxia is the more important goal, if this cannot be achieved, prevention of ensuing irreversible damage may in the future be a realistic possibility. In the meantime, more information about the cerebral haemodynamic and metabolic events that occur during primary and secondary energy failure is urgently needed. One way of obtaining this information is by near infrared spectroscopy, which is discussed below.

Other forms of hypoxic-ischaemic injury

Abnormal phosphorus spectra indicating impaired oxidative phosphorylation and closely resembling those found in babies with birth asphyxia have been found in a variety of other conditions where hypoxic-ischaemic injury was suspected, including periventricular leucomalacia, cerebral infarction, and intraparenchymal periventricular haemorrhage.[10 12 13 26] Also, investigations of babies selected for study because of increased cerebral echodensities suggestive of various types of hypoxic-ischaemic injury have shown that oxidative phosphorylation in brain tissue was quite often impaired.[19 26]

Although detailed information is limited, the temporal progression of the spectral abnormalities in all these conditions seems similar to that which follows birth asphyxia, with the implication that the initiating cerebral insult was often around the time of birth. A further implication (at least theoretically) is that cerebroprotective agents may in the future find a role in preventing or ameliorating permanent ill effects—as in the case of birth asphyxia. Conceivably, antenatal protection may eventually become possible in infants at extremely high risk.

Prognosis

Low [PCr]/[Pi] ratios in infants with increased cerebral echodensities are predictive of the later loss of brain tissue.[19] More recently, a study has been done of the prognostic significance of low values for [PCr]/[Pi] and [ATP] for survival and neurodevelopmental state at the age of 1 year.[13] Sixty one infants born at 27 to 42 weeks' gestation and suspected of hypoxic-ischaemic brain injury were investigated during the first few days of life. Of the 28 infants whose values for [PCr]/[Pi] fell below 95% confidence intervals for normal control infants, 19 died and seven of the nine survivors developed serious multiple impairments (sensitivity 74%, specificity 92% positive predictive value for unfavourable outcome 93%) (fig 5). A significant positive relation was also found between the minimum recorded value for [PCr]/[Pi] and the Griffiths General Quotient at one year.[13] Further, 11 of the 12 infants with low values for [ATP]/[total phosphorus] died and the single survivor had severe multiple impairments (sensitivity 47%, specificity 97%, positive predictive value for death 91%). It was concluded that these data were sufficiently secure that they could in future be taken into account when decisions were made about how far intensive care should be pursued in infants with hypoxic-ischaemic brain injury.

This study also investigated the significance for oxidative metabolism of various different types of cerebral echodensities. It was found that [PCr]/[Pi] was more likely to be abnormally low, and prognosis poor, in infants whose echodensities were diffuse or indicated intraparenchymal haemorrhage (in association with intraventricular haemorrhage) than when they were localised and suggestive of periventricular leucomalacia.[13] In the latter case the lesions were too deep in the brain for abnormalities to be readily detectable by surface-coil magnetic resonance spectroscopy, which acquires signals mainly from the cortex. New developments in the techniques of magnetic resonance spectroscopy will enable this problem to be overcome, and spectra to be obtained from selected volumes of brain tissue.[27]

Near infrared spectroscopy

Near infrared spectroscopy can be used to measure, at the cotside, a range of indices of brain oxygenation and haemodynamics. It thus provides information complementary to that obtained by magnetic resonance spectroscopy, for reasons indicated in fig 6, which gives a simplified account of oxidative phosphorylation.

Electrons are generated by the activity of the citric acid cycle. These pass along the respiratory electron transport chain, which is situated in the inner mitochondrial membrane, and provide an electrical potential that leads to the regeneration of ATP from ADP and Pi. At the end of the chain, the electrons reduce cytochrome aa_3, which then becomes reoxidised by interaction with molecular oxygen. This reaction accounts for about 95% of the oxygen consumption of the body.

Near infrared spectroscopy permits changes in the concentrations in brain tissue of oxyhaemoglobin, deoxyhaemoglobin, and oxidised cytochrome aa_3 to be measured. The data can be processed to give values for various other variables, including cerebral blood flow and blood volume (see below). Information can therefore be obtained about the supply of oxygen to the tissue and (from the oxygenation (or redox) state of cytochrome aa_3) about intracellular oxygen availability.

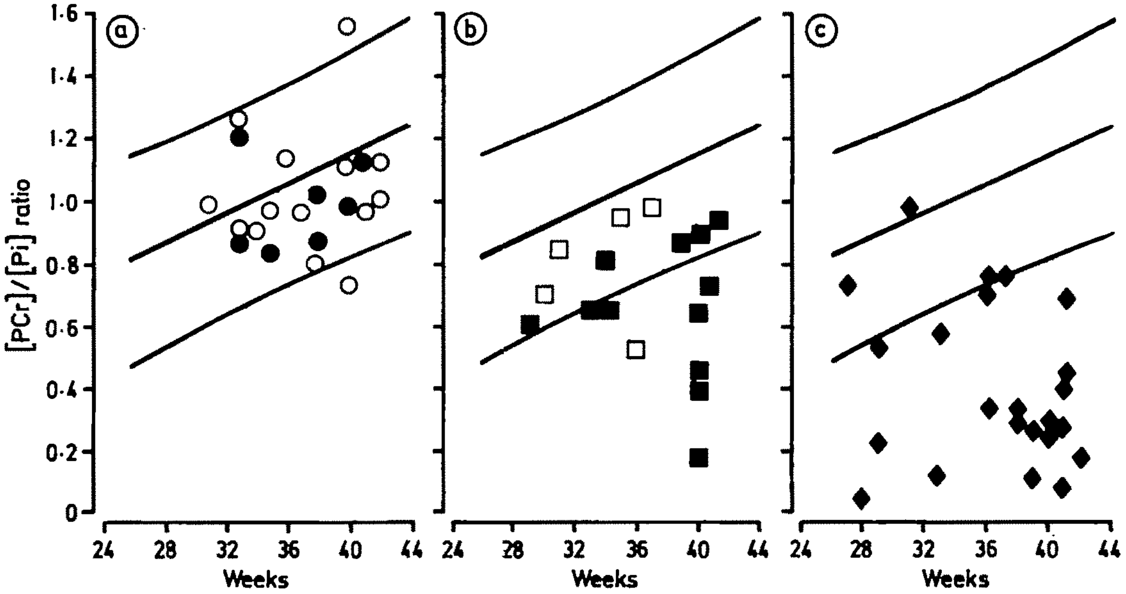

Fig 5 *Relation between minimum values for [PCr]/[Pi] recorded during the first few days of life, survival, and neurodevelopmental outcome aged 1 year in 61 infants suspected of hypoxic-ischaemic brain injury. The regression line and 95% confidence intervals for normal values v gestational plus postnatal age (x axis) are shown. (a) ○=Normal progress at 1 year, and ●=minor impairments (disorders of tone or reflexes not causing disability). (b) □=Major neuromotor impairment causing disability, and ■=multiple major impairments causing disability. (c) ◆=Died. (Data of Azzopardi et al.*[5 13]*)*

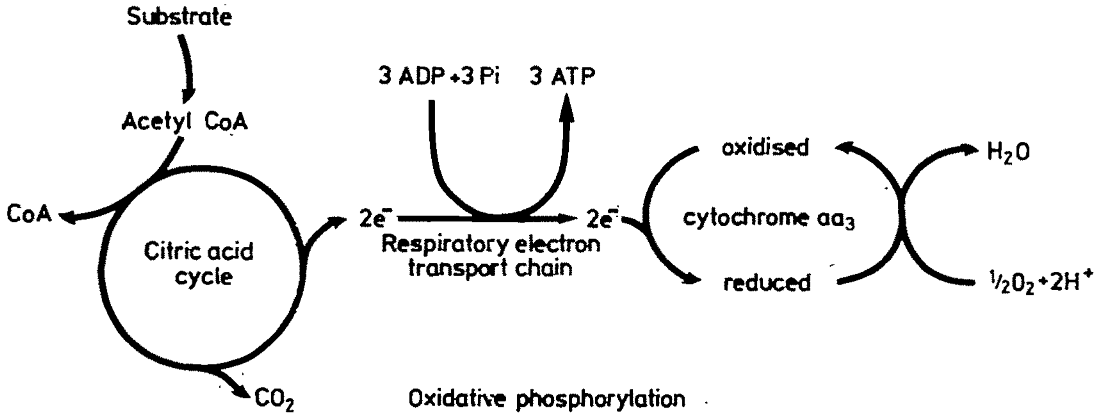

Fig 6 *Diagram showing oxidative phosphorylation.*

The principles behind the technique were first clearly established by Jobsis.[28] They depend on the fact that light in the near infrared region (700–1000 nm) of the spectrum penetrates tissue much more easily than does visible light. With existing technology it is possible to detect near infrared light that has penetrated tissue through distances of about 8 cm, making it possible to perform transmission spectroscopy through all but the largest newborn infant's head. For larger heads observations are still possible by reflectance spectroscopy, though quantitation of the results is not yet so secure.

Oxyhaemoglobin, deoxyhaemoglobin, and oxidised cytochrome aa_3 are chromophores which absorb near infrared light in predictable ways. The absorption characteristics of the tissue therefore give information about the presence of these compounds within it. Brazy *et al* were the first to obtain data from the brains of newborn infants, but quantitation of the compounds was not at that time possible.[29 30]

Quantitative spectroscopy, first performed on newborn infants in 1986,[31] depends on the relation between optical absorption and the concentration of chromophore present. The Beer-Lambert law describing optical absorption may be expressed as: absorption (OD)=(acLB)+G, where OD is optical densities, a is the absorption coefficient of the chromophore ($mM^{-1}cm^{-1}$), c is concentration (mM), L is the distance between the points where light enters and leaves the tissue (cm), B is a 'path length factor' that takes account of the scattering of light in the tissue (which causes the optical pathlength to be greater than L), and G is a factor related to the geometry of the tissue. If L, B, and G remain constant, then changes in chromophore concentration can be obtained from the expression: $\Delta c = \Delta OD/aLB$.

The absorption coefficients of oxyhaemoglobin and deoxyhaemoglobin have been obtained from in vitro studies, and of cytochrome aa_3 from observations from the brain in rats given exchange transfusions of a fluorocarbon blood substitute to remove haemoglobin.[32] The pathlength factor B has been obtained by several different methods,[31-33] including time of flight measurements of an ultrashort pulse of light across the head.[33] These methods gave a value for B of about 4·3 and this is the value now used for quantitation of oxyhaemoglobin, deoxyhaemoglobin, and oxidised cytochrome aa_3 in the brains of newborn infants. In practice, absorption at several different wavelengths is measured and an algorithm is used to convert changes in optical absorption to changes in concentration.[12 31 32] (Absorption by chromophores in extracerebral tissues is negligible.)

OBSERVATIONS IN NEWBORN INFANTS

For the observations to be made, new portable apparatus has been designed and built.[34] Near infrared light at the requisite wavelengths is transmitted into the parietal region through a flexible fibreoptic bundle, and light emerging from the opposite parietal region is collected by an identical bundle and conveyed to a sensitive photomultiplier tube. The distance L is measured with calipers. A controlling computer detects the changes in optical absorption and converts them to changes in chromophore concentration. The results are displayed instantaneously at the cotside and recorded

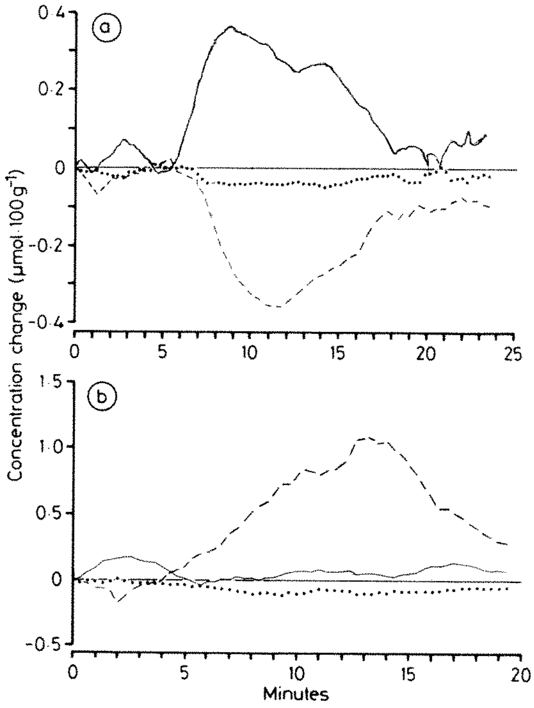

Fig 7 *Changes in the intracerebral concentrations of oxyhaemoglobin (— — — — —), deoxyhaemoglobin (————) and oxidised cytochrome* aa_3 *(. . . . .) in two infants: (a) born at 29 weeks' gestation and studied aged 1 day, and (b) born at 36 weeks' gestation and studied aged 4 days. Both infants had hyaline membrane disease necessitating mechanical ventilation. Changes are shown during: a—alterations in* SaO_2*, which was reduced from 95% to 88% at five minutes and then returned to 95%. b—alterations in* $PaCO_2$*, which increased from 6·1 kPa to 7·2 kPa at 12 minutes, and then fell to 6·2 kPa. As expected alterations in* SaO_2 *were followed by approximately reciprocal changes in oxyhaemoglobin and deoxyhaemoglobin. The increase in* $PaCO_2$ *caused a rise in oxyhaemoglobin but no change in deoxyhaemoglobin, presumably because of an associated increase in cerebral blood flow.*

on computer disk for subsequent analysis.

Alterations in the intracerebral concentrations of oxyhaemoglobin, deoxyhaemoglobin, and cytochrome aa_3 have been quantified in sick newborn infants during small spontaneous or induced changes in arterial oxygen saturation (SaO_2) and $PaCO_2$. Typical results are illustrated in fig 7. The data may then be further analysed to provide absolute values for a number of important variables, including cerebral blood volume, cerebrovascular response to changing $PaCO_2$ and cerebral blood flow.

Cerebral blood volume

Cerebral blood volume may be quantified by observing the effect of a small gradual change in SaO_2 on oxyhaemoglobin and deoxyhaemoglobin.[30] If cerebral haemodynamics do not change during this manoeuvre cerebral blood volume can be calculated from the equation: cerebral blood volume (ml·100 g^{-1})=(Δoxyhaemoglobin−Δdeoxyhaemoglobin)/2(tHb·R·ΔSaO_2) where tHb is the central total haemoglobin concentration (mM·ml^{-1}), and R is the cerebral:large vessel haematocrit ratio (assumed to be 0·69).

Mean cerebral blood volume in 12 preterm and full term newborn infants undergoing intensive care who were thought to have normal brains was 1·7 ml·100 g^{-1}, whereas in 10 comparable infants with brain injury (mostly hypoxic-ischaemic) cerebral blood volume was significantly higher, 3·0 ml·100 g^{-1} (JS Wyatt, M Cope, DT Delpy, *et al*, unpublished observations). The highest values were found in infants who had sustained severe birth asphyxia, presumably because of the effects of vasodilator substances.

Cerebrovascular response to changes in $PaCO_2$

The response of cerebral blood volume to small changes (~1 kPa) in $PaCO_2$ can readily be observed by near infrared spectroscopy. Preliminary studies in infants with normal brains have given values of about 0·5 ml·100 g^{-1}·kPa^{-1}, whereas in infants with birth asphyxia a pronounced reduction or absence of response was found.[35] Fig 8 shows that cerebral blood volume may be increased, and cerebrovascular response to changes in $PaCO_2$ reduced, before the development of secondary energy failure, as detected by magnetic resonance spectroscopy. It is likely that in the future these and other near infrared spectroscopy indices will be useful for identifying infants whose prognosis is particularly bad and in whom the use of new cerebroprotective agents might be tested by controlled trials.

Cerebral blood flow

It has recently been shown that cerebral blood flow can be measured by near infrared spectroscopy[36] using an application of the Fick principle, which states that the rate of accumulation of a tracer substance in an organ is equal to the difference between the rate of arrival and the rate of departure of that substance. When a substance is suddenly introduced into the arterial blood a measurement of the amount accumulated in the organ can be made a a specific time later. If this time is less than the transit time through the organ, the tracer will not appear in the venous blood and flow can be measured as the ratio of tracer accumulated to the quantity of tracer introduced. The near infrared spectroscopy method uses oxyhaemoglobin as the tracer. A sudden change in SaO_2—measured with a pulse oximeter on the ear—is induced by altering the inspired oxygen concentration. The amount of extra oxyhaemoglobin introduced into the brain can then be calculated, and related to the amount accumulated, which is estimated from the change in intracerebral oxyhaemoglobin (fig 9). The expression used to make the calculation is: cerebral blood flow (ml·100 g^{-1}·min^{-1})=(KΔ[oxyhaemoglobin])/([tHb]$_0\int^t$(ΔSaO_2)dt, where K is 0·89, a constant

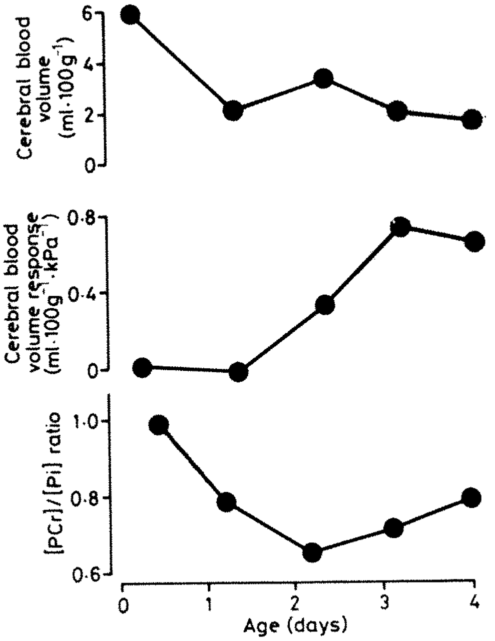

Fig 8 *Cerebral blood volume, its response to small changes in arterial carbon dioxide tension, and [PCr]/[Pi] in an infant with severe birth asphyxia born at 36 weeks' gestation: he developed cerebral atrophy and died aged 7 weeks. Normal ranges are shaded.*

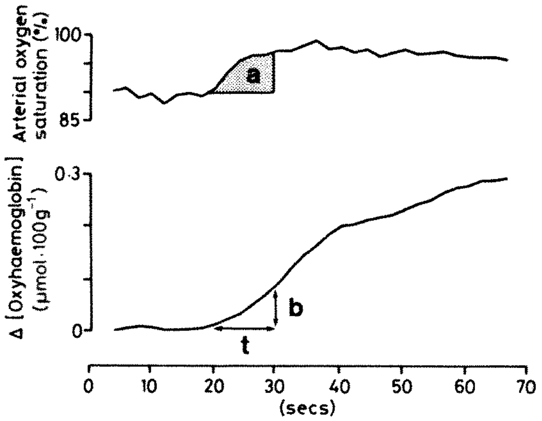

Fig 9 *Method for measuring cerebral blood flow by near infrared spectroscopy. Shaded area (a)* $= {}_0\int^t (\Delta$ *arterial oxygen saturation*$)dt$, *and (b)* $= \Delta$ *oxyhaemoglobin. Terms (a) and (b) were used to derive flow. (From Edwards et al.*[36]*)*

reflecting cerebral to large vessel haematocrit ratio, the molecular weight of haemoglobin and cerebral tissue density; and [tHb] is total haemoglobin concentration obtained from a large vessel.

Thirty one measurements of cerebral blood flow in nine ill, mostly very preterm, infants gave values of about 18 ml·100 g^{-1}·min^{-1}, which appeared comparable with earlier observations made on similar infants using the ^{133}xenon method,[37] or positron emission tomography.[38] Validation of the near infrared spectroscopy method has been performed by making simultaneous measurements of blood flow in the adult forearm by near infrared spectroscopy and by venous occlusion plethysmography.[39] The results corresponded closely.

The effect on cerebral blood flow of the administration of intravenous indomethacin (0·1–0·2 mg·kg^{-1}) for closure of a patent ductus arteriosus has been tested in eight infants.[40] Cerebral blood flow always fell, from a median value of 23 ml·100 g^{-1}·m^{-1} to 9 ml·100 g^{-1}·m^{-1}. Cerebral blood volume and its response to changing $PaCO_2$ and cerebral oxygen delivery, also fell. No recovery of these variables was seen in up to two hours of continuing observations.

Summary and conclusions

Hypoxic-ischaemic injury to the brain is an important cause of perinatal death and seems to be the commonest cause of permanent neurodevelopmental disability in newborn infants who survive after intensive care. If this type of brain injury is to be prevented and treatment put on a rational basis, non-invasive methods are required for defining its mechanisms. This review has considered two such methods: magnetic resonance spectroscopy and near infrared spectroscopy.

Magnetic resonance spectroscopy is used to measure, in brain tissue, the concentrations of the 'high energy' phosphorus metabolites that are dependent for their synthesis on the processes of oxidative phosphorylation. Intracellular pH can also be measured. Normal maturational changes in the brain have been defined and abnormalities detected in a range of conditions where hypoxic-ischaemic injury was suspected to have occurred. In laboratory animals the acute effects of curtailment of oxygen supply to the brain ('primary' energy failure) have been observed, and the effects of two commonly used treatments, infusions of sodium bicarbonate and glucose, have been tested.

After resuscitation of newborn infants from severe intrapartum asphyxia, a latent period has often been noted before energy failure became detectable. This 'secondary' energy failure is due to a variety of damaging reactions initiated by the acute hypoxic-ischaemic episode and reperfusion of the brain. It is possible that in the future irreversible injury to brain cells following the episode may be prevented or ameliorated by the prompt use of cerebroprotective agents.

The extent of abnormalities detected by magnetic resonance spectroscopy has prognostic implications: evidence of severe energy failure in the first days of life was regularly associated with subsequent death or with severe neurodevelopmental impairments.

Many technical developments in magnetic resonance spectroscopy are under way, particularly employing proton (^{1}H) spectroscopy, which will allow the intracerebral concentrations of a wide range of metabolites, including neurotransmitters, to be measured. The combination of spectroscopy with magnetic resonance imaging will permit quantitative data to be obtained from selected volumes within the brain.

Near infrared spectroscopy is used to make observations at the cotside of the intracerebral concentrations of the chromophores oxyhaemoglobin, deoxyhaemoglobin, and oxidised cytochrome aa_3, and it therefore provides information complementary to that obtained by magnetic resonance spectroscopy. Measurements can also be made of cerebral blood flow, cerebral blood volume, and other haemodynamic indices; in addition, the reactivity of the cerebral circulation can be tested. The effects of therapeutic manoeuvres and drugs such as indomethacin can be observed. Near infrared spectroscopy is already providing information about the haemodynamic events that precede and accompany secondary energy failure in newborn infants and in laboratory animals: it seems likely to go into routine use for monitoring brain oxygenation and haemo-dynamics in ill babies, and for guiding treatment.

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References

[1] Alberman ED. The epidemiology of congenital defects: a pragmatic approach. In: Adinolfi M, Benson P, Gianelli F, Seller M, eds. *Paediatric research: a genetic approach.* London: SIMP Heinemann, 1982:1–28.

[2] Cady EB, Costello AMdeL, Dawson MJ, *et al.* Non-invasive investigation of cerebral metabolism in newborn infants by phosphorus nuclear magnetic resonance spectroscopy. *Lancet* 1983;**i**:1059–62.

[3] Younkin DP, Delivoria-Papadopolous M, Leonard J, *et al.* Unique aspects of human newborn cerebral metabolism evaluated with phosphorus nuclear magnetic resonance spectroscopy. *Ann Neurol* 1984;**16**:581–6.

[4] Gadian DG. *Nuclear magnetic resonance and its application to living systems.* Oxford: Clarendon Press, 1982.

[5] Azzopardi D, Wyatt JS, Hamilton PA, *et al.* Phosphorus metabolites and intracellular pH in the brains of normal and small-for-gestational-age infants investigated by magnetic resonance spectroscopy. *Pediatr Res* (in press).

[6] Petroff OAC, Prichard JW, Behar KL, Alger JR, den Hollander JA, Shulman RG. Cerebral intracellular pH by 31P magnetic resonance spectroscopy. *Neurology* 1985;**35**:781–8.

[7] Tofts PS, Wray S. Changes in brain phosphorus metabolites during the postnatal development of the rat. *J Physiol* 1985;**359**: 417–29.

[8] Cady EB, Chu A, Costello AMdeL, *et al.* Brain intracellular pH and metabolism during hypercapnia and hypocapnia in the newborn lamb. *J Physiol* 1987;**382**:1–14.

[9] Hope PL, Costello AMdeL, Cady EB, *et al.* Cerebral energy metabolism studied with phosphorus NMR spectroscopy in normal and birth-asphyxiated infants. *Lancet* 1984;**ii**:366–70.

[10] Hope PL, Reynolds EOR. Investigation of cerebral energy metabolism in newborn infants by phosphorus magnetic resonance spectroscopy. *Clin Perinatol* 1985;**12**:261–75.

[11] Delpy DT, Cope MC, Cady EB, *et al.* Cerebral monitoring in newborn infants by magnetic resonance and near infrared spectroscopy. *Scand J Clin Lab Invest* 1987;**47**(suppl 188):9–17.

[12] Reynolds EOR, Wyatt JS, Azzopardi D, *et al.* New non-invasive methods for assessing brain oxygenation and haemodynamics. *Br Med Bull* 1988;**44**:1052–75.

[13] Azzopardi D, Wyatt JS, Cady EB, *et al.* Prognosis of infants with hypoxic-ischaemic brain injury assessed by phosphorus magnetic resonance spectroscopy. *Pediatr Res* (in press).

[14] Hope PL, Cady EB, Chu A, Delpy DT, Gardiner RM, Reynolds EOR. Brain metabolism and intracellular pH during ischaemia and hypoxia. An in vivo 31P and 1H nuclear magnetic resonance study in the lamb. *J Neurochem* 1987;**49**:75–82.

[15] Hope PL, Cady EB, Delpy DT, Ives NK, Gardiner RM, Reynolds EOR. Brain metabolism and intracellular pH during ischaemia: The effects of systemic glucose and bicarbonate administration studied by 31P and 1H nuclear magnetic resonance spectroscopy in vivo in the lamb. *J Neurochem* 1988;**50**: 1394–402.

[16] Sessler D, Mills P, Gregory G, Litt L, James T. Effect of bicarbonate on arterial and brain intracellular pH in neonatal rabbits recovering from hypoxic lactic acidosis. *J Pediatr* 1987;**111**:817–23.

[17] Laptook AR, Corbett RJT, Nguyen T, Peterson J, Nunnally RL. Alterations in cerebral blood flow and phosphorylated metabolites in piglets during and after partial ischemia. *Pediatr Res* 1988;**23**:206–11.

[18] Siesjo BK. *Brain energy metabolism.* New York: Wiley, 1978.

[19] Hamilton PA, Hope PL, Cady EB, Delpy DT, Wyatt JS, Reynolds EOR. Impaired energy metabolism in brains of newborn infants with increased cerebral echodensities. *Lancet* 1986;**i**:1242–6.

[20] Siesjo BK. Cerebral circulation and metabolism. *J Neurochem* 1984;**60**:883–908.

[21] Schwarcz R, Meldrum B. Excitatory amino acid antagonists provide a therapeutic approach to neurological disorders. *Lancet* 1985;**ii**:140–3.

[22] Chance B, Leigh JS, Nioka S, Sinwell T, Younkin D, Smith D. An approach to the problem of metabolic heterogeneity in brain: ischemia and reflow after ischemia. *Ann NY Acad Sci* 1987;**508**:309–19.

[23] Rothman SM, Olney JW. Excitotoxicity and the NMDA receptor. *Trends in Neurosciences* 1987;**10**:299–302.

[24] McDonald JW, Silverstein FS, Johnston MV. MK-801 protects the neonatal brain from hypoxic-ischemic damage. *Eur J Pharmacol* 1987;**140**:359–61.

[25] Thiringer K, Hrbek A, Karlson K, Rosen KG, Kjellmer I. Postasphyxial cerebral survival in newborn sheep after treatment with oxygen free radical scavengers and a calcium antagonist. *Pediatr Res* 1987;**22**:62–6.

[26] Younkin D, Medoff-Cooper B, Guillet R, Sinwell T, Chance B, Delivoria-Papadopoulos M. In vivo 31P nuclear magnetic resonance of chronic changes in cerebral metabolites following neonatal intraventricular haemorrhage. *Pediatrics* 1988;**82**:331–6.

[27] Hope PL, Blackledge MJ, Ives NK, Rajagopalan B, Sutton P, Radda GK. Spatial localisation of magnetic resonance spectra from neonatal brains. *Pediatr Res* 1988;**24**:264.

[28] Jobsis FF. Noninvasive infrared monitoring of cerebral and myocardial oxygen sufficiency and circulatory parameters. *Science* 1977;**198**:1264–7.

[29] Brazy JE, Lewis DV, Mitnick MH, Jobsis van der Vliet F. Noninvasive monitoring of cerebral oxygenation in preterm infants: preliminary observations. *Pediatrics* 1985;**75**:217–25.

[30] Brazy JE, Lewis DV. Changes in cerebral blood volume and cytochrome aa3 during hypertensive peaks in preterm infants. *J Pediatr* 1986;**108**:983–7.

[31] Wyatt JS, Cope M, Delpy DT, Wray S, Reynolds EOR. Quantitation of cerebral oxygenation and haemodynamics in sick newborn infants by near infrared spectrophotometry. *Lancet* 1986;**ii**:1063–6.

[32] Wray S, Cope M, Delpy DT, Wyatt JS, Reynolds EOR. Characterisation of near infrared absorption spectra of cytochrome aa3 and haemoglobin for the non-invasive monitoring of cerebral oxygenation. *Biochim Biophys Acta* 1988;**933**:184–92.

[33] Delpy DT, Cope M, van der Zee P, Arridge S, Wray S, Wyatt JS. Estimation of optical pathlength through tissue from direct time of flight measurement. *Phys Med Biol* 1988;**33**:1433–42.

[34] Cope M, Delpy DT. A system for long term measurement of cerebral blood and tissue oxygenation in newborn infants. *Med Biol Eng Comp* 1988;**26**:289–94.

[35] Wyatt JS, Edwards AD, Azzopardi D, *et al.* Cerebral haemodynamics during failure of oxidative phosphorylation following birth asphyxia. *Pediatr Res* (in press).

[36] Edwards AD, Wyatt JS, Richardson C, Delpy DT, Cope M, Reynolds EOR. Cotside measurement of cerebral blood flow in ill newborn infants by near infrared spectroscopy. *Lancet* 1988;**ii**:770–1.

[37] Greisen G. Cerebral blood flow in preterm infants in the first week of life. *Acta Paediatr Scand* 1986;**75**:43–51.

[38] Volpe JJ, Hersovitch P, Perlman JM, Raichle ME. Positron emission tomography in the newborn: extensive impairement of regional cerebral blood flow with intraventricular hemorrhage and hemorrhagic intracerebral involvement. *Pediatrics* 1983;**72:** 589–601.

[39] Edwards AD, Reynolds EOR, Richardson CE, Wyatt JS. Estimation of blood flow using near infra-red spectroscopy. *J Physiol* 1989;**410:**50P.

[40] Edwards AD, Richardson CE, Wyatt JS, Delpy DT, Cope M, Reynolds EOR. Effect of indomethacin on cerebral oxygenation and haemodynamics investigated by near infrared spectroscopy. *Pediatr Res* (in press).

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Personal practice

Diagnosis of fetal cardiac abnormalities

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The feasibility of cross sectional imaging of the structure of the fetal heart was first described in 1980.[1–3] Since then fetal echocardiography has been carried out in many pregnancies that are at increased risk of congenital heart disease, and more recently the study of one section of the fetal heart has been included in routine obstetric ultrasound examinations.[4] These studies have yielded surprising results about the incidence, range, and natural history of congenital heart disease that may be seen prenatally. Also the pattern of recurrence of congenital heart disease within family groups does not seem to conform to previously accepted genetic theories.

The mothers who are at increased risk of fetal heart disease include: those with a family history of congenital heart disease; those with diabetes; or those in whom fetal hydrops, arrhythmia, or an extracardiac anomaly has been found. Our most important source of detection of cardiac anomalies in recent years comes from apparently normal pregnancies in which the obstetrician has noticed an abnormal appearance of the heart on a routine scan.

The idea of prenatal cardiac screening came from a French study first reported in 1985 (Fermont L, De Geeter B, Aubry MC, Kachaner J, Sidi D. A close collaboration between obstetricians and cardiologists allows antenatal detection of severe cardiac malformations by 2D echo. Abstract, Second World Congress of Pediatric Cardiology, New York, 1985). A small group of obstetricians was taught to visualise and understand the normal appearance of the four chamber view of the heart and incorporate this into their routine scan (fig 1). When difficulty was experienced in seeing the normal four chamber view the case was referred to a paediatric cardiologist for further evaluation. During a two year period, 20 000 pregnancies were screened, and 39 serious anomalies were detected. This is in accord with the expected figure of 2/1000 that might be detected by this view alone from analysis of data from infants.[5] Screening for congenital heart disease is therefore possible, and it is now being increasingly practised in the United Kingdom. Our success has been less dramatic than the French, because most routine scans in this country are done at 16 weeks' gestation, whereas in France they are done at 20–24 weeks when the fetal heart can be seen more easily. Also some obstetric units in the United Kingdom are handicapped by poor ultrasound equipment or limited time available for adding extra work to the schedule. These problems are slowly being resolved, and we currently have a research programme that is trying to improve the quality and reliability of screening. Although there are still a substantial number of serious anomalies missed by this programme the referral pattern over the years has changed enormously as a result of teaching. Fig 2 shows the increasing number of anomalies detected since 1980, and the proportion of these diagnosed from routine scans.

Although the overall frequency of 2 anomalies/1000 detected by the screening programme is consistent with the expected rate from postnatal

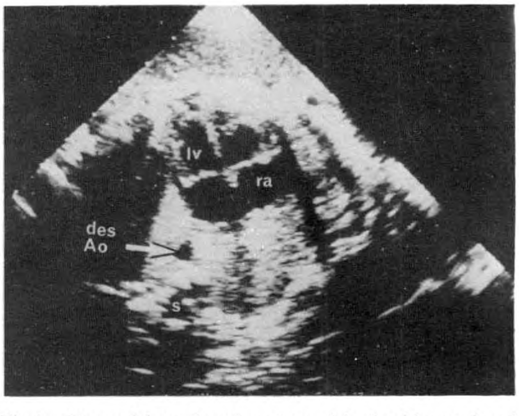

Fig 1 *Normal four chamber view of the fetal heart showing two equally sized atria and ventricles. The heart should occupy about a third of the thorax and the atrial and ventricular septa should meet the atrioventricular valves in a cross at the centre of the heart.*

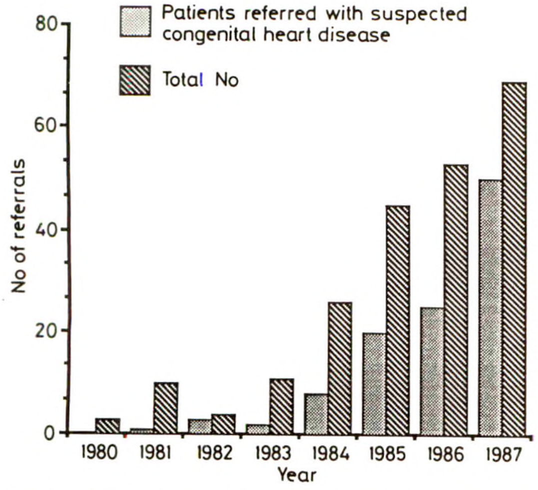

Fig 2 *Total number of detected abnormalities continues to rise, but an increasing proportion is suspected before referral.*

studies, the range of congenital heart disease is different.[6 7] Fig 3 shows a comparison of the diagnostic categories of cases seen prenatally and those reported postnatally. Severe forms of heart disease such as hypoplastic left heart syndrome predominate in prenatal life, and forms of heart disease that are unusual postnatally commonly present in fetal life. Tricuspid dysplasia and the related Ebstein's malformation (fig 4) make up 10% of the anomalies seen in our series. In this condition there is gross tricuspid regurgitation and right atrial dilatation, and there may be associated anatomical or functional pulmonary atresia. The prenatal cardiomegaly restricts lung development resulting in severe pulmonary hypoplasia. Such a neonate is impossible to resuscitate, and dies soon after delivery. Early death accounts for the fact that this condition is rarely encountered in paediatric cardiological practice.

The natural history of some forms of fetal heart disease is unexpected. Many conditions, including hypoplastic left heart syndrome, atrioventricular septal defects, and critical aortic stenosis may present with intrauterine heart failure (fetal hydrops). Spontaneous intrauterine death occurred in 14% of our total series of cases detected prenatally, and this does not include those cases where termination of pregnancy took place in anticipation of intrauterine death. There is a particularly high rate of spontaneous fetal loss in cases of heart disease associated with chromosomal anomalies; the overall incidence in our series of cardiac malformations is 20%. The finding of unusual forms of prenatal heart disease and the high rate of intrauterine death suggest that the incidence of congenital heart disease in early fetal life may be significantly higher than that in postnatal life.

Some abnormalities change or progress during

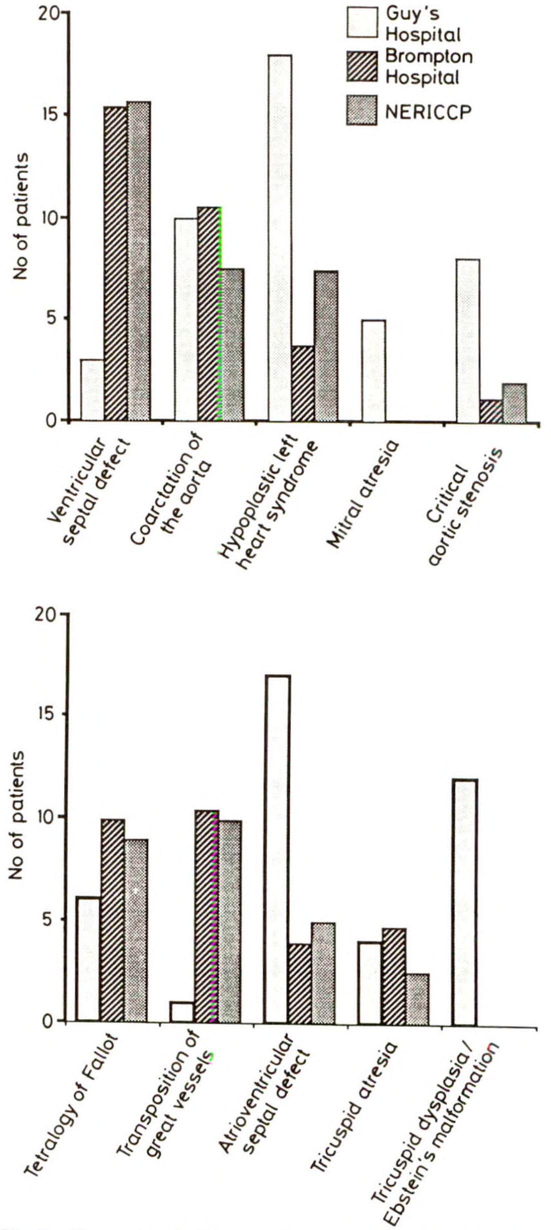

Fig 3 *The range of disease seen in prenatal life in the Guy's unit compared with the scatter seen in infancy reported from the Brompton Hospital, and the New England Regional Infant Cardiac Care Program.*

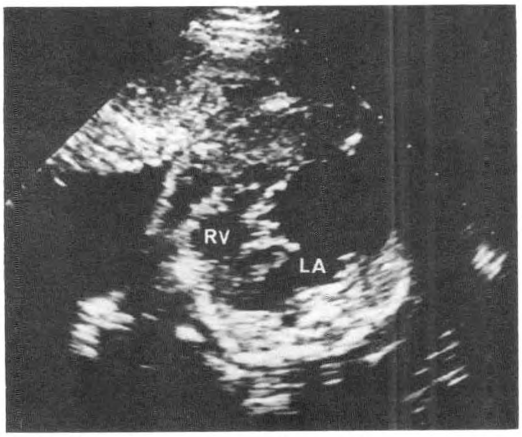

Fig 4 *The heart is enlarged relative to the thorax because tricuspid valve dysplasia and regurgitation causes atrial dilatation, mainly on the right.*

prenatal life. For example, a patient with coarctation of the aorta may develop severe hypoplasia of the aortic arch; the pulmonary arteries in a patient with tetralogy of Fallot may become more hypoplastic; and tumours may enlarge as pregnancy progresses. In several cases the pulmonary valve and right ventricle have looked normal in the early fetal study but serious pulmonary stenosis has developed by full term.

The recurrence of congenital heart disease within a family group seems to depend on the lesion found in the index case, but recurrences do not occur in patterns consistent with generally accepted genetic theories.[8] The overall rate of recurrence is close to the predicted 1/50 but some conditions recur more often than others. The hypoplastic left heart syndrome, coarctation of the aorta, common arterial trunk, and conditions associated with atrial isomerism, are examples of malformations that may recur at a rate of as high as 1/10 when a previous child in the family has been affected. The recurrence rate when a parent is affected is still not clear, but is of the order of 1/10.

The advantage of detection of fetal congenital heart disease

There are several advantages to the prenatal detection of fetal congenital heart disease. The first is to give the parents the option of termination of pregnancy when severe heart disease with a poor long term outlook is found early in gestation. Despite advances in paediatric cardiac surgery there is still a high mortality for many conditions in childhood, before, during, and after operative treatment. The information provided about the heart should be as accurate and objective as possible. The echocardiographer must be aware of the strong association of some forms of heart disease with extracardiac and chromosomal anomalies and take steps to ensure that the parents make decisions with all the possible information available to them. Fig 5 shows an atrioventricular septal defect, which led to the diagnosis of Down's syndrome in the fetus of a young mother. Most parents when faced with the facts of the individual condition found in their fetus choose termination. In our series 75% have taken this step when the diagnosis has been made before 28 weeks' gestation. If screening becomes more widespread there are enormous potential implications for the future practice of paediatric cardiology. Most parents have thought of the possibility of fetal abnormality and how they might react if it happened to them. They already have established views on the morality of abortion in the context of their own lives. Those who have previous experience of congenital heart disease in the family, especially if that child died, are usually particularly anxious to avoid repeating the experience. My attitude is that this decision belongs to the parents alone and that they should be supported whatever decision they make.

Theoretically, when a prenatal diagnosis of heart disease is made and antenatal care is transferred to a unit with combined facilities for obstetrics and paediatric cardiology, the infant should have the optimum chance of survival. Our results, however, do not bear this out, mainly because of the complexity and severity of abnormality selected for presentation for fetal echocardiography, and the

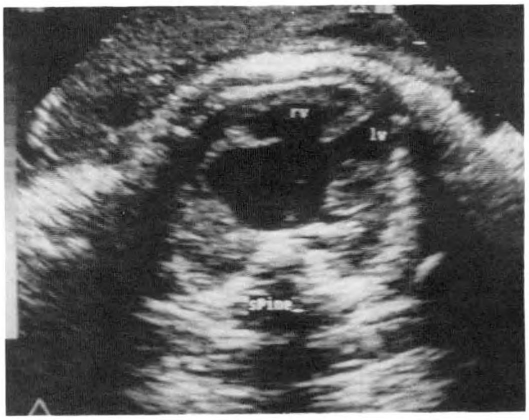

Fig 5 *A large defect in the atrial and ventricular septa at the crux of the heart. There is a common atrioventricular valve, which is open in this diastolic frame.*

incidence of multiple associated extracardiac anomalies. In our series, of 109 babies born alive only 46 survived the first month of life. Of the total series, there are only 22 normal children still alive with either corrected or readily correctable lesions.

Psychological assessment of mothers seen in our department suggests that a mother is emotionally better able to cope with the infant when she has had time to prepare herself prenatally for the problems to be encountered in neonatal life than the mother who learns of her newborn baby's heart disease in the postnatal ward. Many doctors feel that if a mother finds out about a fetal abnormality during pregnancy it will interfere with the experience of pregnancy, but they underestimate most mothers' subconscious and ever present fears of fetal anomaly. Most mothers would elect to have every possible test for the detection of fetal abnormalities if there was no risk attached to the test. The demand for fetal echocardiography has risen from 35 high risk pregnancies in 1980 to over 1000 patients in 1987. The few mothers who do not wish prenatal diagnosis of any kind are able to opt out if they wish.

A further advantage of fetal echocardiography is our increased knowledge of the prevalence and natural history of congenital heart disease in fetal life. This raises the possibility of intervention during prenatal life. It is possible to insert a needle safely into the umbilical cord and even directly into the heart.[9] [10] Will it ever be possible to dilate an arterial valve or burn it with a laser at the end of a catheter prenatally? Would this change the outcome in conditions with a poor prognosis, such as critical aortic stenosis or pulmonary atresia with an intact ventricular septum? Some forms of hypoplastic left heart syndrome have an intact foramen ovale. Would opening this change the course of left ventricular development? These are futuristic concepts but perhaps not so impractical as they first seem. In the United States the time between prenatal diagnosis of the hypoplastic left heart syndrome and delivery is already being used to advantage by seeking a suitable donor heart for transplantation. If this proves to be successful in the long term it may be an avenue to explore in this country. Although I feel that it is fraught with medical, social, and financial problems, some parents are willing to submit their child to any procedure that may give a possible chance of survival. The maximum possible freedom of choice, based on clear, accurate, and unbiased information should be available.

Cardiac function in prenatal life

Most heart disease in infancy and childhood is structural rather than functional. Similarly, disturbed anatomy of the heart is the most common disorder to look for prenatally. Functional anomalies, however, do occur. As in postnatal life, cardiac function can be assessed by looking for peripheral signs of cardiac failure or, more directly, by echocardiography. Cardiac failure in utero is manifest not only by ascites and cardiomegaly as measured by the cardiothoracic ratio but also by pericardial and pleural effusions and skin oedema. These signs can also be caused by non-cardiac lesions.

Cardiac function can also be assessed by Doppler examination of the intracardiac valves. The passive filling phase of the atrioventricular valve tracing will give some indication of ventricular compliance. Atrioventricular valve regurgitation is commonly associated with fetal hydrops. Using a product of the mean velocity of valvar flow and the area of the valve orifice, the volume of blood flow expelled by each side of the heart can be calculated.[11-13] Although this estimation is sometimes inaccurate, it is reasonably reproducible. The output from the right heart is always slightly greater than the left, with a ratio of about 1·3:1. Cardiac output is well maintained until late in the course of fetal compromise[14] but a fall in output indicates a poor prognosis.

Treatment of the fetus

Some possible future invasive interventional techniques have already been mentioned. At present, treatment is confined to giving drugs to the mother to improve the condition of the fetus, but this is mainly for fetal tachycardias.[15] The type of arrhythmia must be documented by M mode echocardiography before treatment is started. An atrial tachycardia without evidence of cardiac failure will usually be converted to sinus rhythm with digoxin alone. For cardiac failure, digoxin and verapamil or flecainide have been successfully used. Premature delivery of a hydropic fetus should be avoided as these infants have a particularly high incidence of necrotising enterocolitis. Once the tachycardia is under control the hydrops can resolve and delivery can take place nearer full term.

In summary, ultrasound examination of the fetal heart can accurately diagnose structural and functional heart disease. It has provided extensive information on the natural history of many forms of congenital heart disease and on normal haemodynamics. There are many possibilities for future developments and applications of the technique.

References

[1] Lange LW, Sahn DJ, Allen HD, Goldberg SJ, Anderson C, Giles H. Qualitative real-time cross-sectional echocardiographic

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Perinatal lessons from the past

Baron Dupuytren (1777–1835) and congenital dislocation of the hip

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Guillaume Dupuytren was one of the ablest doctors in France during the early part of the 19th century. He became surgeon-in-chief at the Hotel Dieu in Paris in 1815. His particular interest was in children's fractures, though perhaps he is best known for his description in 1832 of the contracture of the palmar aponeurosis that was named after him. However, in retrospect, probably his most important contribution was in relation to congenital dislocation of the hip. Although this condition had been discussed in Hippocratic times and mentioned occasionally by other writers, it is to Dupuytren that we owe the first modern account of its anatomy and pathology, its clinical presentation, aetiology, differential diagnosis, and treatment, as the following extracts reveal.[1 2]

'There is a species of displacement of the upper extremity of the femur, of which I have not found any mention in authors . . . This displacement consists in a transposition of the head of the femur, from the cotyloid cavity on to the external iliac fossa (dorsum) of the ilium, a transpostion which exists at birth, and which appears due to a defect in the depth or completeness of the acetabulum, rather than to an accident or disease . . . which I shall name original or congenital dislocation . . . If a practitioner is called in at an early period, to see an infant thus affected, certain indications of this deformity may be detected immediately after birth . . . but, as it almost always happens that any vicious conformation, and the infirmities that spring therefrom do not attract the attention of the parents until the child first attempt to walk, it is at this period that surgeons are, in most instances, called upon to establish the existence of the defect. Then the little patients experience considerable difficulty in walking or running, or even in sustaining the weight of the body in the upright posture; but sometimes it even happens that parents, from carelessness or inattention, imagine that their children are only backward in walking, and do no discover the deformity until three or four years have elapsed.'

Figure *Baron Guillaume Dupuytren of Paris, 1777–1835.*

Dupuytren's classic description of the clinical presentation is remarkably complete and includes the upward and outwards displacement of the head of the femur, the shortening of the affected limb, the prominence of the greater trochanter, the characteristic lumbar lordosis, the telescoping sign, the absence of pain on manipulation, and the distinctive gait. In describing a 9 year old girl with bilateral dislocation he wrote:

'The curvature of the loins, the prominence of the gluteal regions, and the swinging motion of her body when she walked, put me in mind of the waddling of a duck: independently of this, she had very little stability either in standing or walking'.

His attempted treatment of this child is also of great interest:

'The facility with which the limbs could be elongated suggested to us the idea of maintaining such an amount of gentle tension, as to keep the head of either femur on a level with the acetabula: a belt affixed to the hips was employed to press downwards on the upper ends of the thigh bones, whilst traction was employed on the feet for the same purpose, at the same time that the trunk was made fast at its upper part to the bed: in standing or walking the body was always supported on crutches. A certain amount of success encouraged us to persevere in the employment of these measures for a considerable period; but, as our anticipations were not fully realised and the patient was very intractable, the treatment was ultimately abandoned'.

In discussing aetiology Dupuytren dismissed fetal hip disease as a cause, considering abnormal development or actual malformation of the acetabulum more probable. Of particular interest is his discussion of prenatal mechanical factors:

'The position of the lower extremities of the foetus in utero is such, that the thighs are very much bent on the belly, from which it follows that the heads of the thigh bones are continually pressing against the lower and back part of the capsular ligament; a circumstance which, though without effect in well-formed individuals, might, I apprehend, have an injurious influence in such as are weak, or of lax, unresisting fibre. If this premise is conceded, there is not much difficulty in imagining that dislocation may result; and the supposition is further strengthened by the fact that the most powerful of the muscles surrounding the articulation have a constant tendency to draw upwards the heads of the thigh bones, as soon as they escape from the acetabula'.

Dupuytren remarked that congenital dislocation of the hip was 'not so rare as maybe supposed' and that it was frequently bilateral. Furthermore he pointed out that: 'almost all the individuals who are affected with this deformity are females', adding, 'I trust that the observations of others may, at some future period, furnish an interpretation of this phenomenon, and complete that which I have failed to establish in the history of this singular affection'.

References

[1] Dupuytren G. *Memoire sur un deplacement original ou congenital de la tete des femurs.* Paris: Academie de Medicine. 1926.

[2] Dupuytren G. *Injuries and diseases of bones.* Original or congenital displacement of the heads of the thigh-bones. Translated into English by F leG Clark. London: Sydenham Society, 1847:165–86.

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Special report

Recognition and management of fetal abnormalities

MEMBERS OF THE JOINT STUDY GROUP ON FETAL ABNORMALITIES

1 Introduction

1.1 The detection and management of fetal abnormalities are of prime concern to paediatric surgeons because they are responsible for the postnatal care of a substantial number of babies with such defects. In order to encourage cooperation between the specialists involved with the antenatal and immediate postnatal care of the fetus, the British Association of Paediatric Surgeons convened a study group with representation from those colleges and associations whose members would have experience and knowledge in this field. The Department of Health was invited to send an observer who contributed to the discussions. This document has been compiled by the group and its purpose is to improve accuracy in the diagnosis of fetal abnormalities and raise the standard of subsequent care. Encompassed within this objective are aspects which concern doctors in several specialties, nursing and midwifery staff, and providers of health care resources, clinical and laboratory technicians and—most importantly—pregnant women and their families.

The factors are considered under two main headings: diagnosis and management.

1.2 An appendix summarising the results of the Northern Region Fetal Abnormality Survey for 1986[1] is given at the end of the paper. This gives the number and type of fetal abnormalities suspected by routine antenatal screening as a proportion of the number actually born. The number of erroneous diagnoses is also included. These figures illustrate the scale of the problem currently confronting antenatal diagnostic services. Some of the relevant factors were discussed in a previous publication.[2]

2 Diagnosis

2.1 The intention is to outline a standard for screening services for fetal abnormalities for which a number of techniques are available. There is a need for a two tier service comprising district and regional levels with perhaps an additional third supraregional level where intensive, finely focused teaching and research will be carried out and special skills developed.

2.2 All diagnostic units should be able to make immediate assessments of fetal abormalities and be prepared to seek further opinions on diagnosis and treatment from a regional centre. Antenatal ultrasound at district hospital level provides one of the most important diagnostic methods available and its accuracy at the present time is sufficiently good to justify its increasing use.

2.3 We do not have the resources or the expertise to estimate the financial cost of the service we are recommending except to say that extra resources will be required. Neither is it possible to equate the outcome of antenatal ultrasound screening with precise financial gain. It seems clear to us, however, that money for antenatal screening services is very well spent. They can relieve the burden of continuing pregnancy when the fetus is severely malformed, assist in the management of pregnancy, forewarn of some of the impending obstetric complications, give early warning of the need for urgent medical and surgical postnatal care, and offer substantial improvements in maternal and neonatal morbidity.

2.4 It is the duty of the team involved in antenatal diagnosis not only to work closely together so as to create a rational plan of management *but to communicate sympathetically and comprehensively with the parents.* Clearly, the consultant obstetrician or his deputy should be responsible for informing the mother of the results *and implications of the investigations.* Experience indicates that most parents desire antenatal screening for fetal abnormalities.

3 Recommended screening

3.1 Structural malformations of the fetus are a major cause of morbidity and mortality. Screening of all pregnancies is necessary as most (about 80%)

congenital abnormalities occur in the absence of recognised risk factors. Ultrasound examination and measurement of maternal serum α fetoprotein are at the present time the principal means of diagnosis and can achieve a high level of detection of serious abnormalities.

3.2 Structural abnormalities are recognised mainly by ultrasonography and appropriate staff and facilities for ultrasound examination should be available for all pregnant women at an appropriate time in the second trimester.

3.3 With presently available equipment, expertise, and facilities, the minimum gestation at which a detailed fetal scan will provide maximum information is 18–20 weeks. *We believe that normally all pregnant women should be offered a detailed fetal anomaly scan by 20 weeks' gestation.*

3.4 Some centres are offering screening for α fetoprotein to pregnant women at around 16 weeks. If the recommendation in paragraph 3.3 above is accepted, serum screening at 16 weeks will remain important in the earlier detection of neural tube defects. Furthermore, in the light of recent evidence that maternal serum screening at 16 weeks may lead to the recognition of most cases of Down's syndrome,[3] this technique is likely to be employed more widely.

3.5 An explanatory leaflet given to each mother at her first antenatal clinic attendance would be most helpful. *Mothers must be made aware in advance of the limitations and implications of screening and their consent sought.* If the offer of either of the examinations is declined an appropriate comment should be recorded.

4 Pregnancies at high risk of fetal abnormality

4.1 In this numerically small group of pregnancies, management should be tailored to specific needs. This category includes:

(a) Women of 35 years and over.
(b) Fetal abnormality in a previous pregnancy or a poor obstetric history—for example, recurrent abortion, growth retardation, or rhesus haemolytic disease.
(c) A family history of genetic disease identifiable by prenatal diagnostic techniques.
(d) Coexistent maternal disease liable to affect fetal development—for example, diabetes mellitus, hypertension, or renal disease.
(e) Maternal exposure to rubella or other infection liable to affect fetal development.
(f) Maternal drug or alcohol abuse.
(g) Maternal exposure to certain prescribed medications and other suspected teratogens.
(h) Complications during pregnancy—for example, intrauterine growth retardation, oligohydramnios, and polyhydramnios.

4.2 Preconception counselling and early booking for pregnancy care is particularly desirable in such patients. Other techniques may include:

(a) Chorionic villus sampling.
(b) Amniocentesis.
(c) Early anomaly scanning.
(d) Fetal blood sampling.
(e) Fetal tissue biopsy.

5 Clinical management

5.1 Once a fetal abnormality is suspected, a fundamental requirement for good care is close communication among those with diagnostic expertise or potential involvement in neonatal care. This includes neonatologists and specialists in paediatric cardiology, urology, nephrology, neurology, surgery, clinical genetics, cytogenetics, radiology, and fetal/perinatal pathology. All obstetricians in a district or region should have available the names, hospital locations, and telephone numbers of colleagues in the above specialties so that they may be contacted when the need arises.

5.2 The mother and her family should be given the opportunity of a consultation with any of the specialists who will be involved in the management of the pregnancy and postnatal care of the baby. If an abnormality is suspected which will require neonatal surgery, a visit to the neonatal surgical unit where the baby will be nursed postoperatively is helpful.

6 Regional referral centres

6.1 One or more such centres should be made available to each health region to offer training, advice, and support in prenatal diagnosis and clinical management. They should be equipped to provide a full range of prenatal diagnostic techniques including facilities to permit rapid karyotyping when abnormalities are detected in the second three months of pregnancy.

6.2 The main attribute of a regional centre is to bring together the following experts: obstetricians with a special interest in fetal medicine, radiologists practising antenatal ultrasound, neonatologists, clinical geneticists, cytogeneticists and specialists in paediatric surgery, fetal/perinatal pathology, neurology, nephrology, cardiology, and endocrinology.

6.3 It is recommended that a regional advisory committee on fetal abnormalities with members from each of the appropriate specialities be established in each region. This would advise on all aspects of antenatal screening and make recommendations with regard to equipment, staffing, and management. It should make arrangements for collection of data and encourage the development of a regional malformations register. To this register should be added details of anomalies detected in spontaneous abortions and stillbirths.

6.4 Regular meetings of all those involved in the prenatal diagnosis and management of malformations should be set up at regional or subregional level (Area Health Boards in Scotland). It is envisaged that the results of prenatal investigations and detailed pathology findings would be available at such meetings.

6.5 It is recommended that the long term outcome of children with congenital abnormalities diagnosed antenatally be carefully documented.

7 Specific actions

7.1 The first and most important step is to establish as firm a diagnosis as possible. Repeat ultrasound scanning by an appropriately experienced obstetrician or radiologist using more sophisticated equipment may be necessary. Other diagnostic tests such as amniocentesis and fetal blood sampling as detailed in the section on diagnosis may be appropriate.

7.2 Those specialists likely to be involved in the perinatal care of the fetus should be informed so that a plan of management for the remainder of the pregnancy and for the delivery of the baby can be formulated.

7.3 Consultations between the obstetricians, other specialists, and the parents should be arranged at this stage so that the parents understand how the fetus is affected and the options for the further conduct of the pregnancy (see section 8).

7.4 It is important that the team primarily concerned with antenatal care continue to give advice, support, and information even though specialists in other fields become involved. The family practitioner should be kept constantly informed of the patient's progress and consulted over the handling of relevant domestic issues.

8 Alternative courses of action

8.1 TO CONTINUE WITH THE PREGNANCY

Postnatal investigation and treatment are then undertaken as soon as indicated. For some mothers, arrangements should be made for the baby to be delivered in a maternity unit located as close as possible to the regional neonatal surgical unit.

8.2 TO ATTEMPT PRENATAL TREATMENT

Prenatal treatment requires invasive procedures which should be undertaken only in regional centres where the equipment and skilled personnel are available.

8.3 TO TERMINATE THE PREGNANCY

This may be offered for serious fetal abnormalities incompatible with postnatal life and for abnormalities which carry a substantial risk of serious mental and/or physical handicap. It is essential that the mother and her family have the opportunity to discuss every aspect of the problem in full with her obstetrician and all other appropriate experts, to express her wishes, and make an informed choice.

8.4 Adequate provision should be made available for the counselling and care of couples before and after such terminations. Midwives, health visitors, general practitioners, genetic nurses, and voluntary self help groups may all offer valuable support.

9 Fetal pathology

9.1 Each region should have at least one perinatal pathologist. We are aware of the difficulties encountered hitherto and support the Royal College of Pathologists and other interested bodies in pressing for more perinatal pathologists throughout the United Kingdom.

9.2 When a pregnancy is terminated because of a fetal abnormality the fetus should undergo careful examination by a pathologist with an interest and expertise in fetal pathology. If no such expert is available locally the fetus should be transferred to a specialist in perinatal pathology in the appropriate regional centre.

9.3 In appropriate cases, maternal serum and fetal tissue should be examined for TORCH (toxoplasma, rubella, cytomegalovirus, herpes) antibodies and parvovirus. Chromosomes should be examined and a photographic and radiographic record of the fetus should be obtained.

9.4 Products of conception from any pregnancy which aborts after any invasive investigation should be examined by a pathologist (see 4.2).

9.6 The purpose of fetal examination is threefold: firstly, confirmation of the anomaly for which termination was undertaken; secondly, careful scrutiny of fetus, placenta, and membranes for any abnormality which might be related to invasive investigations; thirdly, careful documentation of all abnormalities present to enable a precise diagnosis to be reached so that parents may be advised about the possibility of recurrence.

10 Conclusion

10.1 The proposals set out in this document represent a considerable expansion of the service covering the antenatal diagnosis of fetal abnormalities. The need for additional manpower, training, and equipment will require special study and consultation.

10.2 These recommendations, if implemented, will lead to improvements in the accuracy and sensitivity of the antenatal diagnosis of fetal abnormalities. Better cooperation and more effective exchange of information should enable suspected fetal abnormalities to be managed on a more rational basis.

Appendix

11 The Northern Regional Fetal Abnormality Survey

11.1 This survey of suspected fetal abnormality was started as a pilot study in 1984 and because of its initial success became permanently established in 1985 under the auspices of the Northern Region Health Authority. The structure of the survey and the results for 1986 are available in print from the Northern Region Health Authority.[1] The following data are abstracted from that report with some additional analysis.

11.2 Multiple sources of data collection are employed (table 1) in order to identify major structural malformations presenting in the first year of life. Retrospective assessment of prenatal diagnostic procedures is recorded. In addition, the register records all suspected major malformations and chromosome defects notified through the confidential returns from the region's obstetric units and cytogenetic laboratories. Thus it is possible to record true and false positives and false negatives from a representative population which will provide a measure of the sensitivity and selectivity of existing antenatal diagnostic services.

11.3 ANTENATAL DIAGNOSIS OF SELECTED FETAL ABNORMALITIES 1986
Diagnoses made before delivery in patients delivered in 1986 as a proportion of all cases coming to specialist attention within one year of birth are shown in table 2.

11.4 NEURAL TUBE DEFECT
Almost all anencephalics were identified by the end of the second trimester. Four were not terminated because of a healthy co-twin. Less than half the cases of spina bifida were identified.

11.5 Other anomalies of the central nervous system such as hydrocephalus were diagnosed accurately

Table 1 *Sources of data used to identify major structural abnormalities presenting in the first year of life*

Regional perinatal mortality survey
District health authority SD56 returns
Registrar General's annual death tapes
Laboratories in which α fetoprotein assay is done
District obstetric ultrasound services
Regional cytogenetic laboratories
Records of referrals of patients <1 year from regional centres for:
  Neonatal surgery
  Paediatric cardiology
  Paediatric urology
  Neurology/neurosurgery
Regional genetics advisory service
Grapevine

Table 2 *Diagnoses made before delivery in patients delivered in 1986 as a proportion of all cases coming to specialist attention within one year of birth*

Diagnoses	*No (%) of patients*
Unilateral renal damage treated by nephrectomy	7/7 (100)
Obstruction of pelviureteric junction treated by pyeloplasty	14/14 (100)
Anencephaly	25/27 (93)
Bladder outlet obstruction	7/8 (87)
Hydrocephalus without spina bifida	10/12 (83)
Bilateral renal agenesis or lethal dysplasia	9/13 (69)
Exomphalos/gastroschisis	11/16 (69)
Non-rhesus hydrops	4/6 (67)
Spina bifida with or without hydrocephalus	23/46 (50)
Encephalocele	3/7 (43)
Chylous pleural effusion	1/3 (33)
Duodenal atresia	1/5 (20)
Chromosome abnormality	13/93 (14)
Oesophageal atresia	1/10 (10)
Diaphragmatic hernia	1/16 (6)
Gut atresia below duodenum	0/13 (0)
Lethal skeletal abnormalities	0/4 (0)

but in most at too late a stage in the pregnancy to offer terminaton. Nearly all the survivors of these and other anomalies of the central nervous system are handicapped.

11.6 URINARY TRACT

The accuracy of antenatal diagnosis was good though how many children with unsuspected anomalies presented during the first year of life is unknown at the moment. Two children were identified in 1986 where mild hydronephrosis detected antenatally was not apparent at birth but in whom subsequent investigation showed significant pelviuretic obstruction.

11.7 ABDOMINAL WALL

Nearly 70% of these were correctly diagnosed antenatally with no false positives. Retrospective analysis indicates a substantial improvement in diagnostic sensitivity when compared with the period 1980–3. Prenatal diagnosis permits an examination for associated anomalies and multidisciplinary counselling of parents. If the pregnancy goes to term the mother can be admitted for her delivery to the maternity unit nearest the regional paediatric surgical centre.

11.8 DIAPHRAGMATIC HERNIA

The accuracy of antenatal diagnosis was poor but there is as yet no evidence that prenatal recognition would have improved survival.

11.9 CHROMOSOME ABNORMALITY

Amniocentesis was carried out in only a small proportion of the pregnant women over the age of 35 years. Only six of 52 fetuses (11·5%) with Down's syndrome and 14% of the total number with chromosome anomalies were identified antenatally.

11.10 CONGENITAL HEART DISEASE

Seventy one infants with cardiac malformation delivered in 1986 have been recorded to date but only three were suspected antenatally. Despite subsequent improvements in routine screening for heart defects in this region, it is apparent that the sensitivity of antenatal diagnosis for cardiac malformations remains poor.

11.11 FALSE POSITIVES

This survey is unique in providing information on false positive as well as false negative diagnoses. While antenatal diagnosis was often imprecise there were very few examples of erroneous diagnosis of a serious abnormality. Three suspected cardiac anomalies, one suspected duodenal atresia, one suspected porencephalic cyst, and one spontaneous abortion with suspected spina bifida constituted the principal examples. Caution in interpretation of false positives is necessary, particularly in relation to renal and cardiac defects. The former may not be apparent in the live neonate and cardiac defects may not be recognised without specialist pathological assessment.

11.12 The register contains details of 491 defects, approximately 1·2% of total births in 1986. This figure falls short of the 3% of births associated with congenital anomalies in large epidemiological surveys. In part this is due to incomplete ascertainment of, for example, anomalies which become apparent in later childhood. Furthermore, certain categories such as metabolic defects and isolated non-specific mental retardation have not been included.

11.13 Of recorded cases, 248 (50·5%) received some form of prenatal diagnostic assessment but no abnormality was suspected. Seven (1·4%) received no antenatal screening and in eight (1·6%) no details of the pregnancy were obtained. Abnormality was suspected in 228 (46·4%) but the diagnosis was incomplete or inaccurate in many cases and was often made too late for appropriate intervention.

11.14 A chromosome abnormality imposes a considerable burden on affected families. Rapid chromosome examination when anomaly scanning shows major malformations, together with current developments in the biochemical analysis of maternal serum, have the potential to increase the proportion of chromosomally abnormal fetuses recognised in the second trimester.

References

[1] Northern Region Health Authority. *A regional fetal abnormality survey. First progress report.* Newcastle upon Tyne: Northern Region Health Authority, 1988.

[2] Royal College of Obstetricians and Gynaecologists. *Report of the RCOG working party on routine ultrasound examination in pregnancy.* London: The Royal College of Obstetricians and Gynaecologists, 1984

[3] Wald NJ, Cuckle HS, Densem JW, *et al.* Maternal serum screening for Down's syndrome in early pregnancy. *Br Med J* 1988;**297**:883–7.

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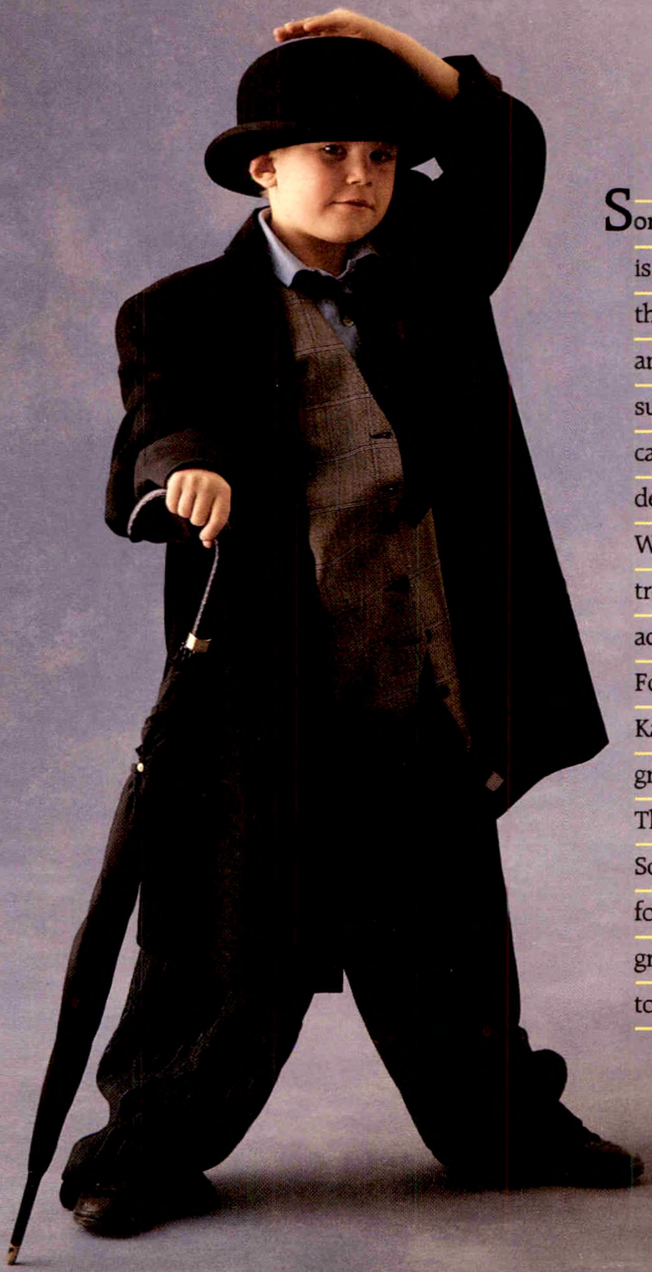

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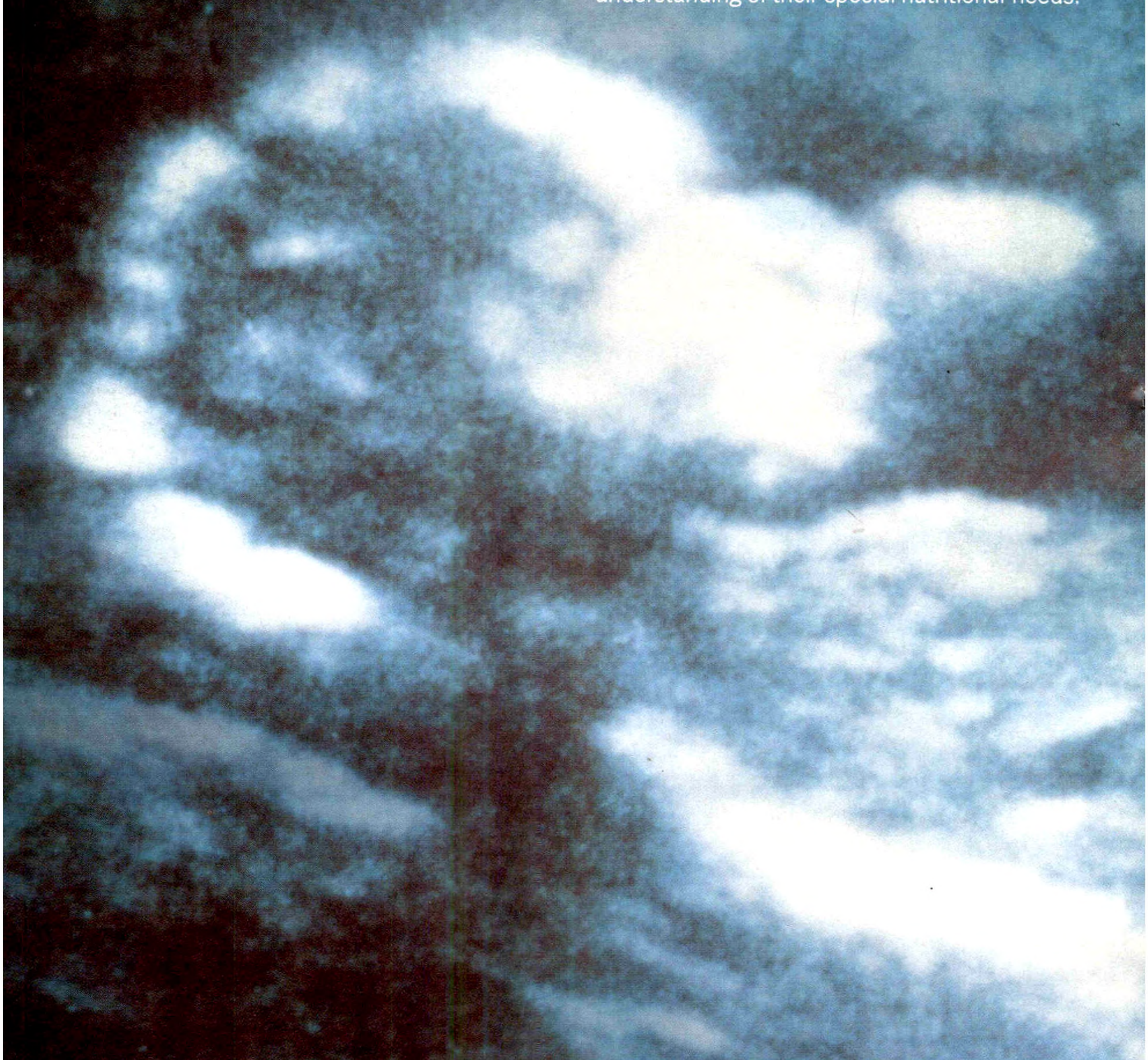

References

1. Committee on Nutrition of the preterm infant. European Society of Paediatric Gastroenterology and Nutrition. Nutrition and feeding of preterm infants. Oxford: Blackwell, 1987.
2. ESPGAN Committee on Nutrition of the Preterm Infant. Acta Paediatr Scand. 1987 Suppl 336: 1-14.
3. Salle, B., Putet G., Service de Neonatologie de L'Hopital Universitaire, Edouard Herriot, Lyon, France. Senterre J., Rigo. J., Service de Neonatologie de L'Hopital Universitaire de Baviere, Liege, Belguim. (Data on file).
4. Mial-Allen V. M., Whitelaw A. G. L. (in press). Comparison of expressed breastmilk versus two low birthweight formulas in the preterm infant. Dept. Paediatrics and Neonatal Medicine, Hammersmith Hospital, London.

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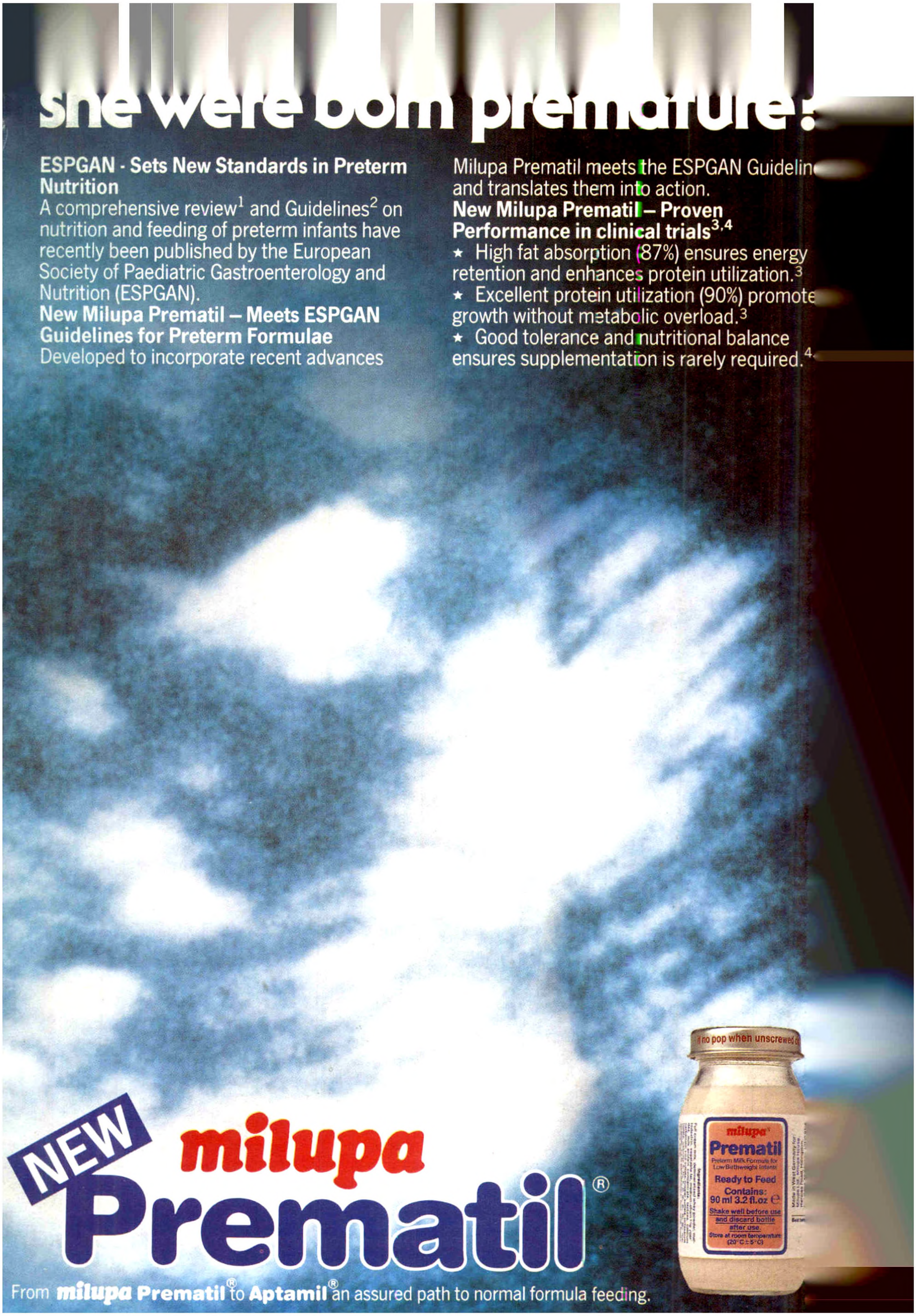
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Editorial

Perinatal nemesis

An epoch in neonatal care was the evolution of assisted ventilation from an experimental technique 30 years ago to a widely used clinical method today. Assisted ventilation and other methods of intensive care have been largely responsible for the rapid improvement in the survival rate of very low birthweight babies in the last 10 years. The price we are paying for our success stories in perinatal medicine is the generation of many new problems and dilemmas. We are faced with a kind of perinatal nemesis (retribution). Illich's notion of 'medical nemesis' was destructive,[1] whereas we in contrast feel that perinatal nemesis should stimulate us to seek new approaches to prevention.

The improved survival of very preterm babies has allowed disorders such as retinopathy, chronic lung disease, and haemorrhagic and ischaemic brain insults to become manifest. It is a moot point whether we class these and other problems as true examples of iatrogenic disease caused specifically by some aspect of treatment, or whether we argue that their origins lie with preterm birth itself. These and other potential causes of disability in surviving preterm babies are subjects of vigorous high quality research which contrasts vividly with the paucity of good quality research into the causes and possible prevention of preterm birth.

'Prematurity' is not a single disease but the culmination of diverse disorders. The socioeconomic factors associated with different patterns of preterm birth require proper appraisal. For example, does the well known social class bias apply equally to mothers with spontaneous onset of preterm labour with intact membranes, and to those with premature rupture of membranes? The notion that 'prematurity' might be prevented by approaches such as preconception counselling, regular attendance at the antenatal clinic, and eating a good [sic] diet continues to be promoted in the virtual absence of any convincing evidence. Informed and good quality preventive research is a high priority if we are to stem perinatal nemesis.

Paradoxically, better and more antenatal care leads to more preterm births by early recognition of the compromised fetus with the option of elective preterm delivery—an option created by paediatricians as a result of the better outcome for preterm babies. The problem of diagnosing fetal growth retardation has been replaced by an even greater challenge to obstetricians—that of the appropriate timing of elective preterm delivery, based on recognition of the threat of antepartum asphyxia. Exciting approaches to the diagnosis of fetal compromise include uteroplacental and umbilical artery flow velocity measurements, and cordocentesis with measurement of blood gases and pH. There is little doubt that we are coming closer to what might turn out to be a key issue, namely the early recognition and possibly protection of the fetal brain from hypoxic/ischaemic and nutritional insults.

The introduction of intrapartum cardiotocography and fetal scalp pH monitoring held the promise of presenting the paediatrician with a baby 'in the best possible condition'. Yet the asphyxiated term baby is not a thing of the past and intrapartum asphyxia can occur unpredictably in spite of scrupulous monitoring. One cause for concern is when, in hindsight, it is considered that the results of monitoring were misinterpreted by the obstetric or midwifery staff. In these cases one sometimes wonders (again with hindsight) whether apparently normal findings on monitoring might have led to complacency in circumstances where the midwife in the pre-technology era would have called for intervention. We argue that this is not an example of technological nemesis, but it should alert us to the need for proper education and training of all staff who work with new technologies whether in the delivery unit or the neonatal intensive care ward.

Advances in molecular biology in terms of the 'new genetics' have opened up avenues for more precise genetic counselling, early antenatal diagnosis, and even treatment of certain genetically determined disorders. Yet we must caution against raising public expectations when in reality so many of the lethal and disabling congenital malformations seen in daily practice occur sporadically, are of uncertain aetiology, and do not currently benefit from genetic innovations. It was inevitable that ultrasound imaging in pregnancy would advance beyond the stage of diagnosing growth retardation and 'pregnancy dating' and extend into the diagnosis of congenital abnormalities. Often an abnormality is discovered late in pregnancy, beyond the time when the pregnancy can be terminated, or the abnormality might not warrant termination of the fetus's life. We accept that sometimes prior warning of certain abnormalities might dictate where the mother should be delivered (if neonatal surgery is likely) or it might suggest the need for further investigations after birth (in renal tract abnormalities). Yet other women who previously enjoyed their pregnancies, blissfully unaware that they were carrying babies with abnormalities of some sort, might now suffer weeks of sorrow and apprehension with no real advantage to the babies over allowing the diagnosis to become apparent naturally after birth. We must not allow parents to suffer the wrath of nemesis. Instead, whenever we offer an ultrasound service with the potential of revealing fetal malformations we must ensure that an obstetrician, paediatrician, or paediatric surgeon is available at short notice to explain the implications of any abnormality in a sympathetic way.

Perinatal nemesis shows itself in diverse ways. The potential for litigation has increased in line with improvements in perinatal care; we are being challenged with ethical dilemmas for which we have had no formal training; we have created a problem of resources in terms of medical and nursing staff, equipment and support services, with each development in perinatal care. Yet we are not so nihilistic as to believe that the story will continue to unfold with one problem replacing another. Perinatal medicine is a comparatively new specialty feeling its way, and it is only natural that here and there nemesis will rain down. One purpose of the Fetal and Neonatal Edition is to encourage a multidisciplinary approach to the specialty and promote good research. It might even help to ward off perinatal nemesis.

Reference

[1] Illich I. *Limits to medicine. Medical nemesis: the expropriation of health.* Harmondsworth: Pelican Books, 1977.

Archives of Disease in Childhood, 1988, **63**, 701–704

Annotations

Prenatal diagnosis of cystic fibrosis

The usual way of diagnosing a genetic disorder in utero is by measuring the concentration of protein product of the mutant gene in an accessible fetal tissue. This is not yet an option for cystic fibrosis. Though the chromosomal localisation of the cystic fibrosis gene is known, and though a 'candidate' gene has been described, the protein product has yet to be identified. Because most of the clinical manifestations of cystic fibrosis are seen in exocrine glands it seems unlikely that the defective protein or enzyme will be expressed in either amniotic fluid cells or in chorionic tissue.

None the less prenatal detection of cystic fibrosis has been possible for several years. One method depends on monitoring early clinical signs of the disorder by measuring a group of microvillar enzymes in amniotic fluid supernatant taken during the second trimester. The other method exploits the tight linkage of an arbitrary set of deoxyribonucleic acid (DNA) markers to the cystic fibrosis gene, and can be applied either to chorionic villus samples taken in the first or second trimester of pregnancy, or to amniotic fluid cells taken during the second trimester. Each method has its advantages and disadvantages; both will probably become obsolete once the cystic fibrosis gene is cloned and sequenced, and the exact mutation defined.

DNA based methods

Late in 1985 several reports[1-3] appeared describing DNA markers linked to the cystic fibrosis gene, which allowed the chromosomal locus to be placed in the region 7q21-34 on the long arm of chromosome 7. A large collaborative study of families collected from Europe and North America[4] showed that in all informative cases the cystic fibrosis gene was linked to a panel of chromosome 7 markers, and therefore in all probability was coded for by the same genetic locus. More recent information using linkage disequilibrium suggested that most European families with cystic fibrosis have inherited the same mutant allele from their ancestors. If this is confirmed, cystic fibrosis may prove to have a formal similarity to sickle cell anaemia and to be a genetic disorder caused by a limited number of mutational events at the same genetic locus.

The panel of DNA probes currently used for the prenatal diagnosis of cystic fibrosis is shown in the table. Most define two allele polymorphisms with the size of fragment depending on the presence or absence of a restriction enzyme cleavage site. Several of the probes may be used with more than one restriction enzyme (for example, pJ3·11, and MspI,[2] or PvuII[5]) to generate additional marker systems. In some cases, however, (for example, pJ3·11, and TaqI, or PvuII) the frequency of the major allele is too high for more than a few individuals to be heterozygous, and these markers are therefore of limited value. The various met probes (pmetH,[1] pmetD,[6] pmet5[5]) are derived from the met proto-oncogene, and others are anonymous DNA fragments. The most suitable probes are pKM·19 and pXV-2c that show few recombinants with the cystic fibrosis gene.[7] The met probes and pJ3·11 have reported recombination fractions of ·004 and ·003, respectively.[4] With p7C22[8] and p79a[9] there are likely to be recombinants several times in every 100 meioses, and such probes should be used with caution in prenatal diagnosis. In practice virtually all families are fully informative when a combination of the most tightly linked probes are used.

In using linked markers to track the cystic fibrosis

Table *DNA probes used in prenatal diagnosis of cystic fibrosis*

Probe	*Restriction enzyme*	*No of alleles*	*Frequency of major allele*	*Recombination fraction with cystic fibrosis*
p79a	MspI	2	0·6	0·05
	HindIII	2	0·7	
p7C22	EcoRI	2	0·75	0·025
pmetH	MspI	3	0·6	0·004
	TaqI	2	0·7	
pmetD	TaqI	2	0·85	0·004
	BanI	2	0·6	
pmet5	TaqI	2	0·7	0·004
	MspI	2	0·9	
pJ3·11	MspI	2	0·5	0·003
	TaqI	2	0·95	
	PvuII	2	0·85	
pXV-2c	TaqI	2	0·5	Very small
pKM·19	PstI	2	0·7	Very small

gene it is always necessary to examine the family in which the disorder is segregating. Normal practice is to take blood samples from both parents and from an index affected child and to establish the phase relation between markers and the cystic fibrosis gene. This is illustrated in the figure for pJ3·11 and the restriction enzyme MspI. Because the index affected child is homozygous for the 1·8 kb DNA fragment on a Southern blot, it can be inferred that this is the fragment in phase with the mutant gene in each of the heterozygous parents. If a chorionic villus biopsy is carried out in the first trimester of a subsequent pregnancy, each of the three possible Southern blot patterns gives unambiguous information on the genotype of the fetus.

The convenient pattern of bands shown on the left hand side of the figure is not usually found with the first marker system tested. One or both parents may be homozygous for the marker, or the index affected child may have the same band pattern as one of the parents. In this case, further marker systems should be tested until either one marker or a combination of markers produces a unique pattern for the index affected child.

It is not usually possible to carry out prenatal diagnosis using linked DNA markers unless there is an index affected child available to establish the phase relations. Unaffected children are unsuitable for typing because it is not possible to tell whether they are homozygous or heterozygous normal. Occasionally it is possible to use an extended family when an affected child on the paternal or maternal side can be used for phase ascertainment.[10] [11] Such typing 'at a distance' increases the chances of an unrecognised recombinant leading to erroneous phase assignments and should not be used if information has to be acquired from the less tightly linked p7C22 or p79a systems.

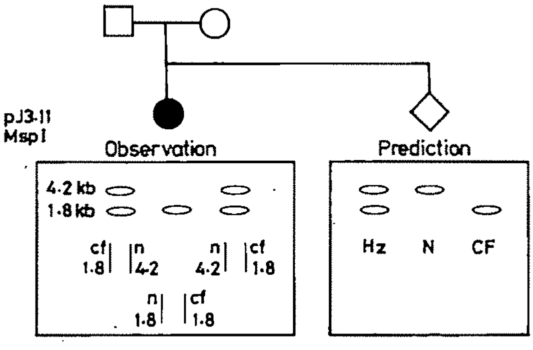

Figure *Schematic representation of use of pJ3·11 and MspI in prenatal diagnosis of cystic fibrosis. Band patterns seen on a Southern blot are shown below parents and index affected child in box (left). The index affected child is homozygous for the 1·8 kb fragment, which is therefore in phase with the cystic fibrosis gene in each parent. Predictions for analysis of a chorionic villus sample are shown in box (right): Hz—heterozygous; N—normal homozygote; CF—affected homozygote.*

Experience of prenatal diagnosis of cystic fibrosis during the first trimester using linked DNA markers has grown rapidly since the first report.[12] Super *et al*[13] described 30 cases; the total from centres in the United Kingdom by December 1987 was 85 (Dr M Super, personal communication). As might be expected there have been some initial difficulties in acquiring adequate amounts of undegraded DNA from occasional over conservative villus biopsy specimens, and in four of the 85 attempts no diagnosis was possible. In seven pregnancies miscarriage followed villus biopsy. In the remaining cases there were no difficulties in either confirming or excluding the presence of cystic fibrosis in the fetus.

Microvillar enzyme testing

In 1983 it was reported that the activities of two peptidases, γ-glutamyltranspeptidase and aminopeptidase M, were significantly depressed in amniotic fluid supernatant during the second trimester in the presence of a fetus with cystic fibrosis.[14] It was subsequently shown that the activities of amniotic fluid disaccharidases[15] and the intestinal isoenzyme of alkaline phosphatase[16] were also greatly reduced. These enzymes have the common feature of localisation on the luminal surface of microvillar membranes, and are found in high concentrations in fetal meconium. It now seems probable that the passage of meconium into amniotic fluid is impaired in the fetus with cystic fibrosis at a critical stage in the second trimester. The primary evidence for this comes from the depleted activities in amniotic fluid of enzymes such as intestinal alkaline phosphatase, which could only originate in the fetal gut. Confirmatory evidence is found in the occasional observations of abnormal ultrasonar scans that suggest a meconium plug in the ileum or jejunum.[17] Pathological examination of the second trimester fetus with cystic fibrosis has now shown clear evidence of extremely abnormal meconium constitution.[18]

In contrast to DNA analysis, measurement of microvillar enzyme is simple, quick, and cheap. Chromogenic substrates are available and assay of a set of enzymes can be carried out on 50 μl of amniotic fluid in less than one hour. Because measurement of microvillar enzyme is monitoring a subtle clinical abnormality in the fetus, however, resolution of normal from abnormal activities in amniotic fluid must be achieved empirically. It must be expected that some fetuses with cystic fibrosis will have microvillar enzyme activities above any arbitrarily defined cut off point (false negatives), and some normal fetuses will be below the cut off point (false positives). Because mothers with apparently affected fetuses will be reluctant to continue their pregnancies, the validation of this system of diagnosis rests to some extent on being able to confirm the prediction in the aborted fetus.

Inspection of fetuses aborted after abnormal microvillar enzyme activity has been reported shows that in most cases the ileum and jejunum are grossly distended and filled with green or black meconium of sticky, glue like consistency.[19] The albumin concentration in the meconium is usually enormously raised. When it is not, there is often a high activity of trypsin like protease.[18] This led to the suggestion that in some fetuses there may have been a burst of pancreatic activity at some point between amniocentesis and termination of pregnancy that could have degraded the more labile intestinal proteins. This idea is supported by the finding of high concentrations of pancreatic oncofetal antigen in meconium from fetuses with normal albumin concentrations and increased protease activities.[20]

Some doubts remain about the validity of using biochemical methods to confirm diagnosis in a cystic fibrosis abortus. The advent of DNA markers has provided an independent method of verification, given that there is a living affected child to establish phase relation. Curtis *et al*[21] showed that adequate amounts of undegraded DNA could be extracted from fetal intestine and other tissues, even after they had been stored frozen for several years. In this way they were able to confirm diagnosis in all eight of the abortuses typed.

It has gradually become apparent that the optimal time for testing for microvillar enzyme activity is at 17 or 18 completed weeks of pregnancy.[22] Earlier amniocentesis leads to a significantly greater false negative rate. Working at this stage of gestation, Boué *et al*[23] reported 200 prospective diagnoses on pregnancies with a 1:4 risk of cystic fibrosis referred to their Paris centre. There was one case incorrectly predicted as abnormal among 142 normal infants (admitted false positive rate 0·7%), and two missed cases of cystic fibrosis among 58 where the diagnosis was an affected fetus (admitted false negative rate 3·4%). These are impressive figures. Much depends, however, on whether all of the 53 terminations of pregnancy were indeed for cystic fibrosis. Of the 43 abortuses examined, all had the biochemical or macroscopic appearance of cystic fibrosis discussed earlier. None the less in this series there were apparently 58 of 200 (29·0%) affected cases, more than expected in a Mendelian recessive condition.

A feature of this study was the concordance between different microvillar enzymes in signalling a normal or affected pregnancy. It has been reported that occasionally extremely low values of γ-glutamyltranspeptidase can be associated with normal activities of other microvillar enzymes.[24] In seven such pregnancies there were six live born babies with cystic fibrosis, and this pattern of activity must be treated with suspicion. Brock *et al*[25] reported a series of 258 pregnancies with a 1:4 risk of cystic fibrosis, in which they found a false negative rate of 4·4% and a false positive rate of 2·3%. Of the pregnancies in their series, however, 32·2% were found or predicted to be abnormal, suggesting a true false positive rate of 8–10%.

If the false negative and false positive rates of tests for microvillar enzyme are estimated conservatively as 5% and 8%, respectively, there are important implications for prenatal diagnosis. For a pregnancy with a 1:4 risk, the chance of a positive test signalling an affected fetus is 80%, and of a negative test signalling a normal fetus 98%. For lower risks, such as when only one partner has a family history, negative tests give extremely high probabilities of normality. Positive tests are ambiguous, however, and in most cases the chance of normality is still greater than the chance of abnormality. For this reason it has been argued that testing for microvillar enzyme activity should be restricted to pregnancies in which the risk is 1:4.[26]

Conclusions

Two different methods of prenatal diagnosis of cystic fibrosis are currently available. The method of choice is diagnosis during the first trimester on a chorionic villus sample using linked DNA probes. The advantages are the high degree of accuracy of diagnosis and the opportunity for termination of pregnancy during the first trimester when the fetus is affected. A disadvantage is the need for a blood or DNA sample from an index affected child in order to establish linkage phase. An alternative form of diagnosis is that testing for microvillar enzyme activity on amniocentesis samples taken during the second trimester. The advantages are the suitability of such testing for all pregnancies with a 1:4 risk of recurrence, and the speed and ease of analysis. Disadvantages are the comparatively high false positive and false negative rates and the fact that if termination of pregnancy is indicated it must be carried out after 18 weeks of pregnancy.

References

[1] White R, Woodward S, Leppert M, *et al*. A closely linked genetic marker for cystic fibrosis. *Nature* 1985;**318**:382–4.

[2] Wainwright BJ, Scambler PJ, Schmidtke J, *et al.* Localization of cystic fibrosis locus to human chromosome 7cen-q22. *Nature* 1985;**318**:384–5.

[3] Knowlton RG, Cohen-Hagenauer O, Van Cong N, *et al.* A polymorphic DNA marker linked to cystic fibrosis is located on chromosome 7. *Nature* 1985;**318**:380–2.

[4] Beaudet A, Bowcock A, Buchwald M, *et al.* Linkage of cystic fibrosis to two tightly linked DNA markers: joint report from a collaborative study. *Am J Hum Genet* 1986;**39**:681–93

[5] Dean M, O'Connell P, Leppert M, *et al.* Three additional DNA polymorphisms in the met gene and D7S8 locus: use in prenatal diagnosis of cystic fibrosis. *J Pediatr* 1987;**111**:490–5.

[6] White R, Leppert M, O'Connell P, *et al.* Further linkage data on cystic fibrosis: the Utah study. *Am J Hum Genet* 1986;**39**:594–8.

[7] Estivill X, Farrall M, Scambler PJ, *et al.* A candidate for the cystic fibrosis locus isolated by selection for methylation-free islands. *Nature* 1987;**326**:840–5.

[8] Scambler PJ, Wainwright BJ, Watson E, *et al.* Isolation of a further anonymous informative DNA sequence from chromosome seven closely linked to cystic fibrosis. *Nucleic Acids Res* 1986;**14**:1951–7.

[9] Estivill X, Schmidtke J, Williamson R, Wainwright B. Chromosome assignment and restriction fragment length polymorphism analysis of the anonymous DNA probe B79a at 7q22 (HMG8 assignment D7S13). *Hum Genet* 1986;**74**:320–2.

[10] Law H-Y, Stanier P, Williamson R, *et al.* Two unusual cases of first trimester prenatal diagnosis of cystic fibrosis using DNA probes. *Prenat Diagn* 1987;**7**:215–21.

[11] Brock D, Curtis A, Holloway S, Burn J, Nelson R. DNA typing to avoid need for prenatal diagnosis of cystic fibrosis. *Lancet* 1986;**ii**:393.

[12] Farrall M, Law H-Y, Rodeck CH, *et al.* First-trimester prenatal diagnosis of cystic fibrosis with linked DNA probes. *Lancet* 1987;**i**:1402–5.

[13] Super M, Ivinson A, Schwarz M. Giles L, Elles RG, Read AP. Clinic experience of prenatal diagnosis of cystic fibrosis by use of linked DNA probes. *Lancet* 1987;**ii**:782–4.

[14] Carbarns NJB, Gosden C, Brock DJH. Microvillar peptidase activity in amniotic fluid: possible use in the prenatal diagnosis of cystic fibrosis. *Lancet* 1983;**i**:329–31.

[15] Van Diggelen OP, Janse HC, Kleijer WJ. Disaccharidases in amniotic fluid as possible prenatal marker for cystic fibrosis. *Lancet* 1983;**i**:817.

[16] Brock DJH. Amniotic fluid alkaline phosphatase isoenzymes in early prenatal diagnosis of cystic fibrosis. *Lancet* 1983;**ii**:941–3.

[17] Papp Z, Toth Z, Szabo M, Szeifert GT. Early prenatal diagnosis of cystic fibrosis by ultrasound. *Clin Genet* 1985;**28**:356–8.

[18] Brock DJH, Bedgood D, Barron L, Hayward C. Prospective prenatal diagnosis of cystic fibrosis. *Lancet* 1985;**i**:1175–8.

[19] Muller F, Frot JC, Aubry MC, Boué J, Boué A. Meconium ileus in cystic fibrosis fetuses. *Lancet* 1984;**ii**:223.

[20] Brock DJH, Barron L. Biochemical analysis of meconium in fetuses presumed to have cystic fibrosis. *Prenat Diagn* 1986;**6**:291–8.

[21] Curtis A, Strain L, Mennie M, Brock DJH. Confirmation of prenatal diagnosis of cystic fibrosis by DNA typing of fetal tissues. *J Med Genet* 1988;**28**:79–82.

[22] Boué A, Brock DJH. Prenatal diagnosis of cystic fibrosis. *Lancet* 1985;**ii**:47–8.

[23] Boué A, Muller F, Nezelof C, *et al.* Prenatal diagnosis of 200 pregnancies with a 1-in-4 risk of cystic fibrosis. *Hum Genet* 1986;**74**:288–97.

[24] Brock DJH, Clarke HAK. An abnormal pattern of amniotic fluid microvillar enzymes signalling fetal cystic fibrosis. *Clin Genet* 1987;**31**:182–5.

[25] Brock DJH, Clarke HAK, Barron L. Prenatal diagnosis of cystic fibrosis by microvillar enzyme assay on a sequence of 258 pregnancies. *Hum Genet* 1988;**78**:271–5.

[26] Brock DJH. Amniotic fluid alkaline phosphatase isoenzymes in early prenatal diagnosis of cystic fibrosis. *Lancet* 1984;**ii**:102.

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Prenatal screening for Down's syndrome

The prevention of non-disjunctional trisomy 21 ('regular' Down's syndrome) depends on offering amniocentesis to older women, and this is not a particularly sensitive screening procedure as the following illustration shows.[1] In 1985 if all pregnancies in England and Wales in women of 35 years or more had been screened (slightly more than 7% of all pregnancies), only 36% of fetuses with Down's syndrome would have been diagnosed prenatally. Fifty one thousand, three hundred and sixty eight amniocenteses would have been performed and about 250 normal fetuses lost, assuming a mortality rate from the procedure of only 0·5%. If one aimed at diagnosing 50% of all fetuses with Down's syndrome one would have to screen all pregnancies in women of 31 and over. This would be unrealistically expensive and too many normal pregnancies would be put at procedural risk.

In practice the uptake of screening because of advanced maternal age falls far short of 100%. For example, in 1984 in the United Kingdom there were only 24 222 prenatal diagnostic chromosome analyses for 705 456 live and stillbirths, or 34·3/1000. Of these 66% were performed because of maternal age of 35 or more, representing only about one third of women in this age group.[2]

More sensitive screening tests are clearly required and may now be available since the publication of studies of the association between fetal Down's syndrome and low maternal serum α fetoprotein concentrations.[3 4] This was confirmed by Wald *et al*[5] in a review of 19 published reports that described a total of 405 pregnancies in which the fetuses had Down's syndrome. More recently there have been similar reports concerning serum unconjugated oestriol concentrations.[6 7] Unconjugated oestriol is a screening test that is said to be independent of maternal age,[6] and largely independent of α fetoprotein concentration, so permitting greater sensitivity. For different combinations of maternal age, maternal α fetoprotein, and oestriol concentrations, a single estimate of risk of Down's syndrome ('isorisk') can be derived. This allows decisions to be taken about the degree of risk judged to be sufficient to warrant offering amniocentesis. For example, to achieve about a 35% detection rate of Down's syndrome (equivalent to amniocentesis for all mothers aged 35 and over) the false positive amniocentesis rate (proportion of unaffected pregnancies having amniocentesis) would be 7·5% if age alone was the criterion, 5·3% using age plus maternal α fetoprotein, but only 2·8% using the combination of oestriol, age, and maternal α fetoprotein. Alternatively, one might decide on a strategy by which a standard investment is made corresponding to a 5% amniocentesis rate. This investment if made in age alone would yield slightly less than 30% detection of Down's syndrome, if made in age and maternal α fetoprotein the yield would be 30–35%, and the best return would be 40–45% from the combination of oestriol, age, and maternal α fetoprotein. Wald *et al*[6] argue the economic advantages of combined screening at a detection rate of 40% because the addition of oestriol to maternal age and maternal α fetoprotein screening would mean that, of every 1000 women screened, 34 (3·4%) would avoid unnecessary amniocentesis resulting in savings greater than the cost of the extra 1000 serum oestriol estimations. Even greater savings would be possible if a simultaneous combined α fetoprotein concentration-oestriol assay were to be developed.

These are important claims, particularly as the impact of maternal α fetoprotein on screening for Down's syndrome has not yet gained general acceptance, and they suggest that there is a need for a review of strategies as more extensive prospective data are accumulated from pilot studies. There will still be false positive results with some unnecessary amniocenteses, although these would be compensated for by the reduction in the number of amniocenteses performed on older women no longer thought to be at high risk. Continuing the existing policy and introducing combined screening would raise amniocentesis rates unacceptably, and a new screening policy not based exclusively on maternal age would exclude some older women. Will obstetricians and patients accept what will seem to be a withdrawal of services when inevitably some older women who would expect amniocentesis on present criteria are found to have low composite risks and nevertheless produce children with Down's syndrome? It will be important to avoid confusion and possible medicolegal problems by clear explanations of new screening criteria. Obstetric teams will have to cope with these problems of re-education, and will need the help of professional counsellors to deal with the greatly increased expectations for patient participation in decisions concerning screening for fetal defects.[8]

The publicity associated with new screening strategies may well increase demand and a 'positive' screening result will lead to greater patient compliance. This will in turn produce more clinical, ultrasonography, laboratory, and counselling work. Facilities available in the United Kingdom for screening for Down's syndrome are patchy, and it is already difficult for most cytogenetic laboratories to meet demand when cash limits are so tight. Biochemistry laboratories will also have to consider the implications on resources as they modify their techniques to make the measurement of low maternal α fetoprotein concentrations more accurate (currently screening concentrates on high maternal α fetoprotein for NTD) and develop assays for unconjugated oestriol concentrations that are suitable for large scale rapid application. Efficient data management and combined estimations of risk are demanding and will include consideration of age, α fetoprotein concentration and oestriol concentrations, and may require further modification for gestational age, maternal weight, and fetal sex. No doubt other screening techniques will be reported and will require assessment, for example fetal femur length.[9] [10] Recently Bogart *et al* have shown that second trimester maternal serum human chorionic gonadotrophin concentrations in pregnancies associated with Down's syndrome may be twice normal.[11] Combining this new marker with maternal age, serum α fetoprotein, and unconjugated oestriol could lead to a Down's syndrome detection rate of 60% for an amniocentesis rate of 5%. Ultimately the use of molecular genetic techniques may permit early prenatal diagnosis by chromosome 21 specific gene probes.

Though there is evidence that a conscientious objection to abortion on the grounds of fetal abnormality is the view of only a minority in our society,[8] personal views must be respected and adequate provision made for survivors with Down's syndrome. These developments and others in fetal screening justify careful and detailed national planning as pilot studies are mounted and before large scale implementation is attempted. The Department of Health and Social Security (DHSS) has been quick to respond to other genetic investigations, and has recently published a favourable report on the work of three centres it funded to evaluate DNA screening.[12] Surely the time has come to establish some form of central forum to advise the DHSS and the research councils on the multidisciplinary problems created by screening for fetal and genetic defects.

References

[1] Brock DJH. Screening for Down's syndrome. *Lancet* 1987;**ii:**1083–4.

[2] ACC Review of Clinical Cytogenetics 1984 England and Wales. London: Association of Clinical Cytogeneticists, 1986.

[3] Merkatz IR, Nitowsky HM, Macri JN, Johnson WE. *Am J Obstet Gynecol* 1984;**148:**886–94.

[4] Cuckle HS, Wald NJ, Lindenbaum RH. Maternal serum alpha-fetoprotein measurement: a screening test for Down's syndrome. *Lancet* 1984;**i:**926–9.

[5] Wald NJ, Cuckle HS. Recent advances in screening for neural tube defects and Down's syndrome. *Obstetrics* 1988 (in press).

[6] Wald NJ, Cuckle HS, Denselm IW, *et al*. Maternal serum unconjugated oestriol as an antenatal screening test for Down's syndrome. *Br J Obstet Gynaecol* 1988 (in press).

[7] Canick JA, Knight GJ, Palomaki GE, Haddow JE, Cuckle HS, Wald NJ. Low second trimester maternal serum unconjugated oestriol in prenancies with Down's syndrome. *Br J Obstet Gynaecol* 1988 (in press).

[8] King's Fund Forum: screening for fetal and genetic abnormality. *Br Med J* 1987:**292:**1551–3.

[9] Benacerraf BR, Gelman R, Frigoletto FD. Sonographic identification of second trimester fetuses with Down's syndrome. *N Engl J Med* 1987;**317:**1371–6.

[10] Lockwood C, Benacerraf BR, Krinsky A, *et al*. A sonographic screening method for Down's syndrome *Am J Obstet Gynecol* 1987;**157:**803–8.

[11] Bogart MH, Pandian MR, Jones OW. Abnormal maternal serum chorionic gonadotrophin levels in pregnancies with fetal chromosome abnormalities. *Prenat Diagn* 1987;**7:**623–30.

[12] Department of Health and Social Security. Special medical development in clinical genetics, interim report: clinical effectiveness in the service context. *Medical Division CDPNM* London: DHSS, 1987.

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Maternal and fetal β endorphin: effects of pregnancy and labour

The recent discovery of specific opiate receptors in the brain and the pituitary gland and of endorphins that bind to these receptors led to many studies on the role and effects of these substances. The pituitary gland secretes β endorphin at the same time as β lipotrophin, and adrenocorticotrophic hormone (ACTH).[1]

β endorphin in pregnancy and labour

Various workers have measured peripheral plasma β endorphin concentrations in women during pregnancy, labour, and delivery. Their reported absolute β endorphin concentrations and their conclusions about whether or not pregnancy is associated with a rise in maternal plasma β endorphin concentrations, are at considerable variance. The differences in absolute β endorphin concentrations may be largely due to differences in methods and antibody specificity.

Goland *et al*[2] measured maternal plasma β endorphin concentrations during pregnancy. They reported marginally but not significantly higher concentrations in pregnant women. When measuring plasma concentrations in relatively large groups of non-pregnant controls and first, second, and third trimester pregnancies, we found a significant nadir during the second trimester (table 1).[3] Genazzani *et al*[4] reported a significant decrease in maternal plasma β endorphin at 9–12 weeks' gestation and an increase near full term (36–37 weeks' gestation) when compared with those found in non-pregnant controls. These discrepancies may be attributed to the selection of study subjects, the number of patients studied, and the stringent classification of subjects.

In our study venous blood samples from pregnant and non-pregnant volunteers were taken between 8 and 10 am, and none of the study subjects was to undergo any surgical operation or induction of labour unless stated otherwise. The increase in maternal plasma β endorphin concentrations in women at full term reported by Genazzani *et al*[4] was because their subjects were about to undergo elective caesarean section. Our data indicated that the phychological stress that is associated with preparation for a major operation such as caesarean section without premedication is enough to induce increased pituitary release of β endorphin.[3] Thus, one may conclude that in the absence of specific stress, maternal plasma β endorphin is decreased rather than increased throughout pregnancy until labour ensues.

During labour maternal plasma β endorphin concentrations rise and remain high during the early postpartum period (table 1). This is most consistent with the increase in secretion of ACTH that has been reported to occur during labour and to peak at delivery.[5 6] Csontos *et al*[7] reported parallel increases in maternal plasma β endorphin and ACTH concentrations. Our observation that maternal plasma β endorphin concentrations remain raised for some

Table 1 *Plasma β endorphin concentrations in non-pregnant women, women during pregnancy and labour, 30–60 minutes postpartum, and just before elective caesarean section in the absence of labour*[3]

Subjects	*No of samples*	*Mean (SE) β endorphin concentration (pg/ml)*	*p Value (Mann-Whitney U test) compared with non-pregnant controls*
Non-pregnant control subjects	17	58 (2·4)	
Women in first trimester	11*	47 (2·4)	<0·005
Women in second trimester	11*	33 (1·9)	<0·0005
Women in third trimester	10*	49 (2·7)	<0·02
Women in early labour (cervical dilatation <4 cm)	12	202 (32·0)	<0·00005
Women in advanced labour (cervical dilatation >4 cm)	10	389 (78·0)	<0·00005
Women 30–60 minutes postpartum	12	177 (22·0)	<0·00005
Women awaiting caesarean section	15	151 (23·0)	<0·005†

*Measurement on plasma pooled from four volunteers, representing 44, 44, and 40 subjects, respectively; †compared with women in the third trimester.

time after delivery, despite the short half life of β endorphin,[8] indicates that the maternal pituitary continues to secrete increased amounts of β endorphin after delivery.

Plasma β endorphin concentrations fall in response to epidural analgesia during labour and delivery,[9-12] and after intrathecal morphine analgesia during labour.[13] The decline in maternal plasma β endorphin concentrations after maternal analgesia while uterine contractions and progress in labour continue indicate that pain, stress associated with pain, or both, are major stimuli of pituitary β endorphin release rather than labour itself. These data have been validated by the use of appropriate controls in whom epidural catheters were placed but saline rather than an anaesthetic agent was initially injected.[9] This significant decline in circulating β endorphin is probably due to decreased pituitary secretion of β endorphin in response to the alleviation of the stress induced by labour pain, and is consistent with the short half life of β endorphin measured as 4·1, 13·1, and 46·2 minutes, respectively, with its multiexponential disappearance curve.[8]

β endorphin and the fetus

Plasma β endorphin concentrations are a measure of stress not only in the mother but also in the fetus. β endorphin concentrations rise significantly in response to fetal distress (table 2). This increase of β endorphin in the fetal circulation in response to stress was shown to be paralleled by a concomitant rise in beta lipotrophin,[14] a finding that is consistent with the fact that β endorphin originates from β lipotrophin by selective cleavage. Wardlaw *et al*[15] showed a significant inverse correlation between umbilical plasma β endorphin concentrations and PaO_2 and pH, indicating that fetal hypoxia or acidosis, or both, may be related to β endorphin release. Data have shown that umbilical venous plasma β endorphin concentrations are higher than umbilical arterial plasma β endorphin concentrations as measured in 22 paired samples without apparent fetal distress,[3] suggesting that the placenta contributes to the pool of circulating fetal β endorphin. In the presence of fetal distress, however, umbilical arterial β endorphin concentrations seem to rise more extensively than umbilical venous concentrations, suggesting that the fetal pituitary is capable of secreting β endorphin in response to stress.

This conclusion is consistent with the findings of Fachinetti *et al* that β endorphin is present in the plasma of newborn babies during the first 24 hours of life.[16] Considering that β endorphin has a short half life,[8] the data of Fachinetti *et al*[16] seem to indicate that the fetus born at full term is capable of producing β endorphin, probably by release from the pituitary. That corticotrophin releasing hormone secreted in response to stressful stimuli may not only initiate the selective cleavage of ACTH but also that of β endorphin from their common precursor pro-opiomelanocortin in the fetus and newborn, seems to be an attractive hypothesis. Hypoxia may be the overriding stress stimulus in the fetus, and opioid peptides in the fetal central nervous system may act as neurotransmitters that modulate fetal heart rate patterns and decrease fetal heart rate variability.[17] It has been shown that neither the mode of anaesthesia,[11 18] presence or absence of labour, nor the mode of delivery, affect umbilical vein plasma β endorphin concentrations.[3] The latter finding contradicts that reported by Raisanen *et al* in which normal vaginal delivery without asphyxia increased β endorphin release into the fetoplacental circulation.[19]

Table 2 *Umbilical venous plasma β endorphin concentrations in newborn babies delivered by various routes and modes with and without apparent intrapartum fetal distress*[3]

Route and mode of delivery	*Fetal distress*	*No of samples*	*Mean (SE)β endorphin concentration (pg/ml)*	*p Value (Student's t test) compared with spontaneous vaginal delivery*
Spontaneous vaginal delivery	No	14	128 (19·8)	
Elective caesarean section	No	12	131 (20·5)	NS
Emergency caesarean section	No	18	109 (18·1)	NS
Forceps or vacuum delivery	No	10	161 (31·4)	NS
Forceps or vacuum delivery	Yes	6	366 (59·7)	<0·01
Emergency caesarean section	Yes	4	421 (138·0)	<0·05
Combined groups without fetal distress	No	54	133 (11·1)	
Combined groups with fetal distress	Yes	10	398 (62·4)	<0·001*

*Compared with the combined groups without fetal distress.

References

[1] Guillemin R, Vargo T, Rossier, *et al.* β-endorphin and adrenocorticotrophin are secreted concomitantly by pituitary gland. *Science* 1977;**197:**1367.

[2] Goland RS, Wardlaw SL, Stark RI, Frantz AG. Human plasma β-endorphin during pregnancy, labour and delivery. *J Clin Endocrinol Metab* 1981;**52:**74–8.

[3] Goebelsmann U, Abboud TK, Hoffman DI, Hung TT. Beta-endorphin in pregnancy. *Eur J Obstet Gynecol Reprod Biol* 1984;**17:**77–89.

[4] Genazzani AR, Facchinetti F, Parrini D. β-lipotrophin and β-endorphin plasma levels during pregnancy. *Clin Endocrinol* 1981;**14:**409–18.

[5] Allen JP, Coor DM, Kendall JW, McGilvra R. Maternal-fetal ACTH relationship in man. *J Clin Endocrinol Metab* 1973;**37:**230–4.

[6] Winters AJ, Oliver C, Colston C, MacDonald PG. Plasma ACTH levels in the human fetus and neonate as related to age and parturition. *J Clin Endocrinol Metab* 1974;**39:**269–73.

[7] Csontos K, Rust M, Hollt V, Mahr W, Kromer W, Teschemacher HJ. Elevated plasma β-endorphin levels in pregnant women and their neonates. *Life Sci* 1979;**25:**835–44.

[8] Reid RL, Hoff JD, Yen SSC, Li CH. Effects of endogenous β-endorphin on pituitary hormone secretion and its disappearance rate in normal subjects. *J Clin Endocrinol Metab* 1981;**52:** 1179–84.

[9] Abboud TK, Sarkis F, Hung TT, *et al.* Effects of epidural anesthesia during labor on maternal plasma beta-endorphin levels. *Anesthesiology* 1983;**59:**1–5.

[10] Thomas TA, Fletcher JE, Hill RG. Influence of medication, pain and progress in labor on plasma β-endorphin-like immunoreactivity. *Br J Anaesth* 1982;**54:**401–8.

[11] Browning AJF, Butt WR, Lynch SS, Shakespear RA, Crawford JS. Maternal and core plasma concentrations of β-lipotrophin, β-endorphin and γ-lipotropin at delivery; effect of analgesia. *Br J Anaesth* 1983;**90:**1152–6.

[12] Raisanen I, Paatero H, Salminen K, *et al.* Pain and plasma β-endorphin level during labor. *Obstet Gynecol* 1984;**64:**783.

[13] Abboud TK, Goebelsmann U, Raya J, *et al.* Effects of intrathecal morphine analgesia during labor on maternal plasma beta endorphin levels. *Am J Obstet Gynecol* 1984;**149:**709–10.

[14] Shaaban MM, Hung TT, Hoffman DI, Lobo RA, Goebelsmann U. β-endorphin and β-lipotrophin concentrations in umbilical cord blood. *Am J Obstet Gynecol* 1982;**144:**560–8.

[15] Wardlaw SL, Stark RI, Baxil L, Frantz AG. Plasma β-endorphin and β-lipotrophin in human fetus at delivery. Correlation with arterial pH and PO_2. *J Clin Endocrinol Metab* 1979;**49:**888–91.

[16] Fachinetti F, Bagnoli F, Bracci R, Genazzani AR. Plasma opioids in the first hours of life. *Pediatr Res* 1982;**16:**95–8.

[17] Goodlin RC. Naloxone and its possible relationship to fetal endorphin levels and fetal distress. *Am J Obstet Gynecol* 1981;**139:**16–19.

[18] Jouppila R, Jouppila P, Karlquist K, Kaukoranta P, Leppa-Luoto J, Vuolteenaho O. Maternal and umbilical venous plasma immunoreactive β-endorphin levels during labor with and without epidural analgesia. *Am J Obstet Gynecol* 1983;**147:** 799–802.

[19] Raisanen I, Paatero H, Salminen K, Laatikainen T. β-endorphin in maternal and umbilical cord plasma at elective cesarean section and in spontaneous labor; *Obstet Gynecol* 1986;**67:**384.

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Changes in fetal acid base status during intravascular transfusion

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SUMMARY Umbilical venous pH, PCO_2, PO_2, and base excess were measured immediately before and after 72 intravascular transfusions in 34 fetuses with erythroblastosis fetalis. In 67 uncomplicated transfusions, infused adult blood led to a mean (95% confidence intervals) fall in pH (0·037, CI 0·029 to 0·044) and base excess (2·03, CI 1·61 to 2·45) and a mean rise in PCO_2 (0·24 kPa, CI 0·13 to 0·35). These changes correlated significantly with the increase in fetal haemoglobin and packed cell volume. Five transfusions were associated with complications within six hours: intrauterine death in two, fetal distress necessitating delivery in two, and preterm labour in one. Two had pre-existing acidosis, whereas two of the three with normal blood gas and acid base measurements before transfusion had acute changes that were outside the normal ranges that had been established in uncomplicated transfusions.

During recent years direct access to the fetal circulation has not only established a role for the intravascular transfusion of anaemic fetuses with erythroblastosis fetalis,[1-3] but has also permitted closer study of the fetal environment in rhesus isoimmunisation.[4-6] Antenatal intravascular transfusion, whether performed fetoscopically,[1 7] or by needling under ultrasonographic control,[3 8-11] carries risk to the fetus. The total risk of the procedure is the risk of fetal blood sampling[12] plus the risk of transfusion. Several reasons for fetal death after transfusion have been proposed, including changes in fetal oxygenation, and the direct cardiotoxic effects of the anticoagulant in donor blood.[7]

Normal ranges for fetal blood gas and acid base measurements have been described[13] and used as a basis for intervention in compromised pregnancies.[14 15] This study describes the normal ranges for changes in fetal blood gas and acid base measurements during uncomplicated transfusions, and assesses their use in predicting complications associated with transfusions.

Methods

Seventy two intravascular transfusions were performed for severe rhesus isoimmunisation by needling of the umbilical vein under ultrasonographic control either in the intrahepatic portion or at the placental cord insertion. Thirty four fetuses, including one set of dichorionic twins, underwent one to four procedures at intervals of two to five weeks at gestational ages of 19 to 33 weeks. The technique, volume, and rate of transfusion have been described elsewhere.[3] Before transfusion, the packed cell volume and haemoglobin concentration of each fetus were determined by a Coulter S Plus counter (Coulter Electronics Ltd, Luton, England), and an additional 250–300 µl of fetal blood was aspirated into a heparinised syringe for blood gas analysis. An intravenous rather than intra-arterial position of the needle was confirmed by the direction of flow of transfused blood seen on ultrasound scan. Donor blood was adult group O, Rh negative, and seronegative for cytomegalovirus. It had been collected within the previous 24 hours, crossmatched against maternal blood, and made up to a packed cell volume of 0·65 to 0·79. After the infusion the needle was flushed with 1 ml of normal saline and 1 ml of blood was aspirated and discarded. Samples for repeat haematological investigations, and a heparinised specimen for blood gas analysis were then taken. As in similar investigative studies, the additional volume of blood required was not considered to increase the risk to the fetus,[6] especially as transfusion was replacing the aspirated volumes.

Blood gas analysis was performed within 20 minutes with an ABL 330 (Radiometer, Copenhagen, Denmark). Base excess was calculated from pH and PCO_2 using the Thews and Harnoncourt nomogram to correct for haemoglobin concentration. Blood gas analysis was also performed on heparinised samples from nine donor packs immediately before use.

All haemoglobin concentrations before transfusion were ≥40 g/l, below which pronounced acidosis occurs.[5 6] Sixty seven transfusions in 32 fetuses were uncomplicated and the pregnancies allowed to continue. Five procedures in five singleton fetuses, however, were complicated by delivery or death of the fetus within six hours of transfusion. One of these had ascites, as did 11 of the 67 uncomplicated procedures.

Fetoplacental volume was calculated using the formula $y=22\cdot34+0\cdot44x^2$ where x is gestational age.[16] Changes in variables after transfusion are expressed as the post-transfusion value minus the pre-transfusion value. The normal distribution of changes in blood gas measurements was confirmed with histograms. Changes within transfusions were analysed statistically by the two tailed paired Student's *t* test, whereas comparisons between transfusions were considered as independent samples. Normal 95% data intervals were calculated from the formula: mean plus or minus 1·96 × the standard deviation. Associations with haematological and obstetric data were assessed by Pearson's correlation coefficient.

Results

In 67 uncomplicated intravascular transfusions the mean pH and base excess fell, there was a rise in mean PCO_2, but the PO_2 did not change significantly (table 1). Significant, albeit weak, negative correlations were found between the changes in haematocrit and changes in pH ($r=-0\cdot39$ $p<0\cdot01$) and the change in base excess ($r=-0\cdot34$, $p<0\cdot01$), and similarly between the changes in haemoglobin concentrations and changes in pH ($r=-0\cdot34$, $p<0\cdot01$) and changes in base excess ($r=-0\cdot336$, $p<0\cdot01$). Changes in both packed cell volumes and haemoglobin concentrations correlated positively with changes in PCO_2 ($r=0\cdot323$, $p<0\cdot01$, and $r=0\cdot345$, $p<0\cdot05$, respectively). There was no significant correlation of the acid base or blood gas changes with gestational age or volume transfused, except a weak positive correlation between the change in PCO_2 and transfused volume expressed as a percentage of fetoplacental volume ($r=0\cdot260$, $p<0\cdot05$).

To determine any effect of adult haemoglobin on blood gases, the changes in values in the initial transfusions (when the haemoglobin before transfusion is entirely fetal) were compared with subsequent transfusions when fetal blood may contain predominantly adult type haemoglobin (table 1). The drop in pH was slightly greater ($p=0\cdot045$) during the initial transfusions, but changes in PCO_2, PO_2, and base excess were similar. In addition, there was no significant difference in changes in pH, PCO_2, PO_2, or base excess between tranfusions in fetuses with pre-existing ascites and transfusions in those without ascites.

Donor blood had a mean (range) pH of 6·76 (6·57–6·94), PCO_2 of 16·82 kPa (15·7–17·9), and PO_2 of 3·96 kPa (3·25–4·67).

Five transfusions were associated with complications within six hours (table 2). There were two intrauterine deaths caused by the procedure. Fetal asystole occurred immediately after the procedure in case 1, responded at first to intracardiac and intramuscular adrenaline (0·5 ml of 1/100 000 at each site), but recurred within 15 minutes. Fetal pH at the time of fetal cardiac injection was 6·55. Transfusions in two patients (Cases 3 and 4) were complicated by fetal distress necessitating delivery. Fetal bradycardia occurred during the procedure in case 3 and was followed by a baseline tachycardia with late decelerations. Cord pH at caesarean section was 7·19. Case 4 developed variable decelerations in the presence of normal Doppler

Table 1 *Mean (95% CI) changes in blood gas and acid/base measurements in uncomplicated transfusions for, all first, and subsequent procedures*

	All procedures (n=67)	*First procedures (n=27)*	*Subsequent procedures (n=40)*
pH	−0·037* (−0·029 to −0·044)	−0·047† (−0·031 to −0·061)	−0·030† (−0·021 to −0·038)
PCO_2	0·24* (0·13 to 0·35)	0·33 (0·10 to 0·55)	0·18 (0·07 to +0·29)
PO_2	0·06 (+0·23 to −0·01)	0·29 (−0·07 to +0·65)	−0·10 (+0·06 to −0·27)
Base excess	−2·03* (−1·61 to −2·45)	−2·53 (−1·71 to −3·36)	−1·69 (−1·26 to −2·12)

*=p<0·001; †=p<0·05 (first compared with subsequent procedures).

Table 2 *Five procedures in which complications occurred within six hours*

Case No	*Gestation (weeks)*	*Procedure No*	*pH before transfusion*	*Complications during procedure*	*Complications after procedure*
1	26	2	7·42	Bradycardia	Intrauterine death within 15 minutes
2	32	2	7·38	None	Intrauterine death within three hours
3	32	5	7·37	Transient bradycardia	Fetal distress; emergency caesarean section at four hours
4	34	2	7·24	None	Variable decelerations; emergency caesarean section at six hours
5	30	3	7·20	None	Ruptured membranes; spontaneous delivery within four and a half hours

waveforms on testing of the umbilical artery. At 34 weeks, with borderline pre and post-transfusion pH, and with an increase in fetal ascites after the first transfusion, the decelerations were considered adequate grounds for delivery. Case 5 had persistent hydramnios and hydrops for six weeks. Although she did not complain of contractions, her membranes ruptured and she was fully dilated within three hours of the procedure. She was considered in retrospect to have been in labour at the time of transfusion, this being the probable cause of the low fetal pH measurements (7·20 before, and 7·17 after the transfusion).

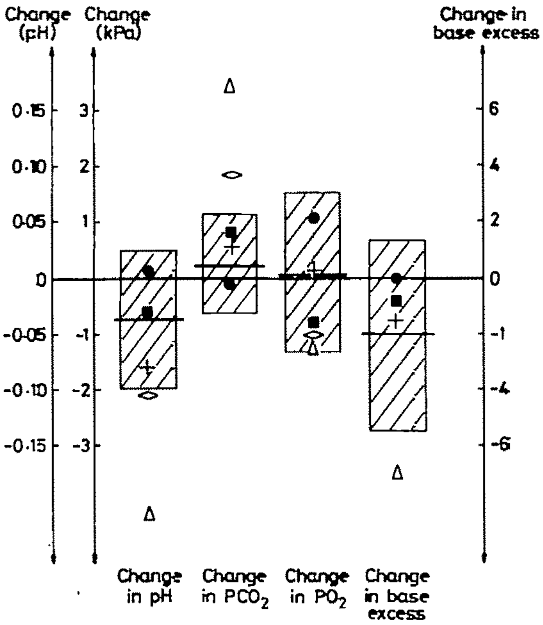

Figure *Changes in pH, PCO_2, PO_2, and base excess in complicated transfusions expressed against normal ranges for uncomplicated transfusions. Patient 1=<>. 2=†, 3=△, 4=●, 5=■. In patients 4 and 5 fetal acidosis was present before transfusion started.*

The figure shows a comparison of the changes in blood gas and acid base measurements in five complicated transfusions, with normal ranges established in uncomplicated transfusions. Two of the three procedures with a normal pH before transfusion, were associated with abnormal changes for pH, PCO_2, and base excess. No result for base excess was available in case 1 because of a fault in the machine.

Discussion

Transfusion of packed cells in human fetuses produced an acute fall in pH and base excess, and a rise in PCO_2. This drop in pH and base excess was the result of infusion of exogenous acids in donor blood rather than vascular spasm leading to hypoxaemia because there was no concomitant decrease in PO_2.

Given the degree of acidosis in donor blood (mean pH 6·76), the changes were minor due to the buffering of acidotic donor blood during fetoplacental circulation. A suggested advantage of transfusion into the umbilical artery instead of the umbilical vein as used in this study, is that before reaching the fetus the acidotic blood is buffered and oxygenated during passage through the placental circulation.[2 3] Lactic acid was found earlier in samples from the umbilical artery than from the umbilical vein in patients with severe rhesus disease, consistent with a substantial placental capacity for buffering.[5] In this study donor blood injected into the umbilical vein must have undergone one, and presumably several, fetoplacental circulations to affect acid base measurements in fetal blood collected at the same site. To determine the relative buffering capacities of fetus and placenta, simultaneous samples from both umbilical artery and vein would be required after transfusion into either vessel. Such a study would pose ethical and technical difficulties.

Given the extremely high PCO_2 concentrations in donor blood, the rise in PCO_2 during transfusion is only small, again due to the buffering capacity of the placenta. The increase in PCO_2 and the fall in base excess together determine a tendency towards mixed respiratory and metabolic acidosis.

The lack of significant change in PO_2 reflects similar PO_2s in donor and fetal blood. An increase in PO_2 might be expected in the presence of pre-existing hypoxia. There is, however, considerable evidence that the anaemic fetus maintains adequate tissue oxygenation until the haemoglobin concentration falls below 40 g/l[4-6] and in this study no haemoglobin concentration was below 40 g/l. Chronic rises in umbilical vein PO_2 after transfusion have been reported in human[17] and animal fetuses,[18 19] but may represent a compensatory rise in uteroplacental flow secondary to tissue hypoxia.[17]

The change in pH in first transfusions was slightly greater than in subsequent transfusions (−0·047 and −0·030, p=0·045), but the difference in base excess was not significant. These findings are consistent with haemoglobin type having only a comparatively minor effect on blood gas and acid base measurements despite the greater affinity with oxygen of fetal compared with adult haemoglobin.[17]

Pre-existing fetal acidosis in two transfusions associated with complications suggested that the condition before transfusion rather than the intrauterine transfusion was responsible for delivery within six hours. In the remaining three complicated transfusions, two (cases 1 and 3) were associated with abnormally large changes in pH, PCO_2, and base excess suggesting an adverse reaction beginning during the procedure. The change in PO_2, however, was within normal limits, pointing to compromise of fetoplacental perfusion rather than of placental exchange. Although both fetuses developed bradycardia during infusion this was non-specific, occurring also in several uncomplicated transfusions. Intrauterine death three hours after transfusion in the remaining patient (case 2) was unexpected and unassociated with intraoperative complications or blood gas and acid base abnormalities. Necropsy was not performed. Although intraperitoneal transfusion may cause a fetal vagal bradycardia after the procedure, none of the five in which complications developed underwent the combined procedure of intravascular transfusion followed by intraperitoneal transfusion which has become our practice in order to prolong intervals between transfusions (Nicolini *et al*, unpublished observations).

Acute or subacute fetal distress or death, or both, after fetal blood sampling may be due to haematoma of the cord producing tamponade, fetomaternal haemorrhage, or exsanguination into the amniotic cavity. Additional mechanisms for acute or subacute complications after transfusion are poorly understood. Mackenzie *et al* reviewed the possibilities and considered it unlikely that the cardiotoxic effects of citrate anticoagulant, potassium, and adenosine release from donor blood were responsible.[7] The degree of acidosis in donor blood does not seem to be important. They used blood buffered to a pH of 7·20–7·35 and noted bradycardia during transfusion in six of 10 fetuses, eight of whom did not survive the perinatal period. This is in contrast to the paucity of complications in our series in which we used unbuffered blood.

This description of acute changes in acid base and blood gas parameters after transfusion in human fetuses permits calculation of normal ranges to predict complications directly related to the procedure. Closer postoperative monitoring of those with abnormal values who are likely to produce a live fetus may prevent fetal death by allowing timely intervention such as occurred in Case 3. We recommend the routine measurement of pH, PCO_2, and base excess before and after intravascular transfusion.

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References

[1] Rodeck CH, Holman CA, Karnicki J, Kemp JR, Whitmore DN, Austin MA. Direct intravascular fetal blood transfusion by fetoscopy in severe rhesus isoimmunisation. *Lancet* 1981;**i:**625–7.

[2] Rodeck CH, Nicolaides KH, Warsof SL, Fysh WJ, Gamsu HR, Kemp JR. The management of severe rhesus isoimmunization by fetoscopic intravascular transfusions. *Am J Obstet Gynecol* 1984;**150:**769–74.

[3] Nicolaides KH, Soothill PW, Rodeck CH, Clewell W. Rh disease: intravascular fetal blood transfusion by cordocentesis. *Fetal Therapy* 1986;**1:**185–92.

[4] Nicolaides KH, Rodeck CH, Millar DS, Mibashan RS. Fetal haematology in rhesus isoimmunusation. *Br Med J* 1985;**290:**661–3.

[5] Soothill PW, Nicolaides KH, Rodeck CH, Clewell WH, Lindridge J. Relationship of fetal hemoglobin and oxygen content to lactate concentration in Rh isoimmunized pregnancies. *Obstet Gynecol* 1987;**69:**268–71.

[6] Soothill PW, Nicolaides KH, Rodeck CH. Effect of anaemia on fetal acid-base status. *Br J Obstet Gynaecol* 1987;**94:**880–3.

[7] Mackenzie IZ, Bowell PJ, Ferguson J, Castle BM, Entwhistle CC. In utero intravascular transfusion of the fetus for the management of severe Rhesus isoimmunisation—a reappraisal. *Br J Obstet Gynaecol* 1987;**94:**1068–73.

[8] Bang J, Bock JE, Trolle D. Ultrasound guided fetal intravenous transfusion for severe rhesus haemolytic disease. *Br Med J* 1982;**284**:373–4.

[9] De Crespigny LCh, Robinson HP, Quinn M, Doyle L, Ross A, Cauchi M. Ultrasound-guided fetal blood transfusion for severe Rhesus isoimmunization. *Obstet Gynecol* 1985;**66**:529–32.

[10] Grannum PA, Copel JA, Plaxe SC, Scioscia AL, Hobbins JC. In utero exchange transfusion by direct intravascular injection in severe erythroblastosis fetalis. *N Engl J Med* 1986;**314**:1431–4.

[11] Berkowitz RL, Chitkara U, Wilkins I, Lynch L, Mehalek KE. Technical aspects of intravascular intrauterine transfusions: lessons learned from thirty three procedures. *Am J Obstet Gynecol* 1987;**157**:4–9.

[12] Daffos F, Capella-Pavlovsky M, Forestier F. Fetal blood sampling during pregnancy with use of a needle guided by ultrasound: a study of 606 consecutive cases. *Am J Obstet Gynecol* 1985;**153**:655–60.

[13] Soothill PW, Nicolaides KH, Rodeck CH, Campbell S. Effect of gestational age on fetal and intervillous blood gas and acid base values in human pregnancy. *Fetal Therapy* 1986;**1**:166–75.

[14] Nicolaides KH, Soothill PW, Rodeck CH, Campbell S. Ultrasound guided sampling of the umbilical cord and placental blood to assess fetal wellbeing. *Lancet* 1986;**i**:1065–7.

[15] Pearce JM, Chamberlain GVP. Ultrasonically guided percutaneous umbilical blood sampling in the management of intrauterine growth retardation. *Br J Obstet Gynaecol* 1987;**94**:318–21.

[16] Nicolaides KH, Clewell WH, Rodeck CH. Measurement of human fetoplacental blood volume in erythroblastosis fetalis. *Am J Obstet Gynecol* 1987;**157**:50–3.

[17] Soothill PW, Nicolaides KH, Rodeck CH, Bellingham AJ. The effect of replacing fetal with adult haemaglobin on blood gas and acid/base parameters in human fetuses. *Am J Obstet Gynecol* 1988;**158**:66–9.

[18] Battaglia FC, Bowes W, McGaughey HR, Makowski EL, Meschia G. The effect of fetal exchange transfusions with adult blood upon fetal oxygenation. *Pediatr Res* 1969;**3**:60–5.

[19] Meschia G, Battaglia FC, Makowski EL, Droegemuller W. Effect of varying umbilical blood O_2 affinity on umbilical venous pO_2. *J Appl Physiol* 1969;**26**:410–16.

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Costs and benefits of neonatal intensive care

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SUMMARY A cohort of very low birthweight infants (<1500 g) born in 1979–81 from a geographically defined area was followed up, and a costing of the initial admission to the neonatal intensive care unit was made. A four point scale for the severity of disability was used and estimation of the costs of education and full time residential care was made and discounted at 5% over the appropriate period. During the three years a progressively increasing proportion of infants survived, and this was associated with an increasing proportion of disabilities among the survivors. If costs are related to outcome up to the age of 4 they get progressively less over the three year study period. After the age of 4 the costs of special education and residential care dominate, and the cost trend is reversed.

Mortality rates among low birthweight infants have shown a particularly sharp decline in the past decade and at least some of this is attributable to modern methods of neonatal intensive care.[1-3] Higher survival rates are, however, also associated with increased costs and a greater incidence of impairment.

Relatively little is known about the long term costs of caring for low birthweight infants but a detailed clinical follow up has made it possible to assess survival, disability, and morbidity in a geographically defined population. Detailed costing of a neonate intensive care unit has been combined with a study of the costs of health care up to the age of 4 and a projection of long term costs for the care of the disabled. This had allowed a comparison to be made of the costs and benefits of neonatal intensive care.

Health economists have proposed that costs and benefits may be neatly combined in the concept of the cost of a quality adjusted life year (QALY) gained.[4] This is one measure of efficiency and it has been applied in the evaluation of neonatal intensive care units in Canada.[5] In this study we present estimates of the cost of producing QALYs in a neonatal intensive care unit over a three year period during which there was a considerable change in clinical practice following the appointment of a consultant neonatologist.

Methods

A cohort of very low birthweight infants (VLBW) (<1500 g) born during 1979–1981 to residents in two health districts on Merseyside (Liverpool and South Sefton) who had at least one admission to the neonatal intensive care unit were studied. They were followed up, and examined when they were between 3 and 4 years old to see what disabilities they had and what use they had made of medical services. They are a subgroup of a larger cohort of which details of history, examination, and clinical assessment have been previously described.[6]

DISABILITIES

A four point scale for severity of disability was used:

1. Normal—no clinically apparent neurodevelopmental abnormality causing functional disability.
2. Mild disability—for example, myopia, language delay, mild hearing loss, or hyperactivity.
3. Moderate disability—for example, diplegia, hemiplegia, or moderate learning disability (intelligence quotient 50–69).
4. Severe disability—for example, quadriplegia, blindness, deafness (loss 70 decibels or more in either ear), hydrocephalus, uncontrolled epilepsy, or severe learning disability (intelligence quotient <50).

Those children in class 4 were assumed to have a life expectancy at the age of 5 of 40 years. Those in classes 1, 2, and 3 were assumed to have a normal life expectancy of 70 years.

COSTS

Details of the clinical management of the infant's initial admission to the neonatal intensive care unit until the time of its first discharge were obtained. If the infant was transferred to any other specialist unit during this initial period (for example, for operation) it was included in the costing of the initial admission. The degree of care during this admission was subdivided into: intensive care—infants receiving respiratory support either by intermittent positive pressure ventilation or by constant positive airways pressure; special care—infants receiving either electronic monitoring or intravenous infusions, or both, but not requiring respiratory support; and nursing care—infants receiving special observation or care but fed orally and not requiring respiratory support or intravenous infusions.

All survivors were followed up until the age of 4 to record all admissions for inpatient care and all outpatient visits. A detailed costing of the initial admission to the neonatal intensive care unit was carried out, details of which have been previously described.[7]

A projection was made for disabled children of the likely costs of special education and institutional care over their expected life span. It was assumed that infants with disabilities of group 2 severity would incur no costs of this sort. Children with disabilities groups 3 or 4 severity were assumed to require special education from the age of 4 to 19 years and institutional care from the age of 19 until death. This strong assumption reflected the view that even if some of these disabled children and adults received part of their care at home, they would impose on their families costs similar in magnitude to the cost of institutional care. Costs were derived from information provided by the Liverpool Education and Social Services Departments. A deduction was made for the cost of educating a normal child.

A further adjustment was made for differences in the quality of life. The children were allocated a quality of life coefficient according to the severity of their disability. A child that died scored zero and a normal child was assigned a score of one. The remaining groups were scored at 0·75, 0·50, or 0·25 according to whether they had been assessed as mildly, moderately, or severely disabled. Expected life spans were multiplied by the coefficients to give an estimate of the QALYs that each infant might expect. For instance, 40 years of life for a severely disabled person were taken as equivalent to 10 years of normal life. This crude method of adjusting for the quality of life would not be acceptable in a study which included larger numbers of disabled infants. In this study because the numbers were small the findings were shown not to be sensitive to the choice of coefficients.

All costs were expressed in accordance with National Health Service pay and prices in 1984. Costs and benefits that are spread over many years have to be reduced to a common base to allow comparisons to be made. Following the normal procedure they have been discounted at 5%. Discounted costs can be thought of as the capital sum that, invested at 5% in 1984, would produce a stream of income sufficient to purchase care for the cohort over its lifetime.

Results

Of 157 infants in the cohort five were lost to follow up. The data apply to the 152 children for whom follow up records were available.

A progressively increasing proportion of infants survived in the three years of the cohort but this was associated with an increasing incidence of disability among the survivors. These outcomes with adjustments for life expectancy and the quality of life are shown in table 1.

Table 2 shows costs of neonatal intensive care divided into initial and later hospital costs, and the lifetime costs of special education and residential care. The cost of care for those who died is included. In the 1979 cohort only one child had a disability that was of group 2 severity, and by assumption he did not incur any further costs for special education or

Table 1 *Outcomes for 152 VLBW infants 1979–81*

	1979	*1980*	*1981*
No of infants	43	53	56
No of survivors (%)	19 (44)	29 (55)	40 (71)
No of disabled survivors	1	6	5
No of quality adjusted lives	18·75	25·5	37·0
No of QALYs	1313	1760	2572
No of QALYs discounted	363·6	492·6	714·4

Table 2 *Long term costs (£) of neonatal intensive care at 1984 prices for 152 VLBW infants. 1984 prices discounted at 5%*

	1979	*1980*	*1981*
Initial hospital admission	254 815	278 239	346 108
Later hospital admission and outpatient attendance to age 4	23 015	68 280	139 290
Subtotal: hospital costs to age 4 (early hospital costs)	277 830	346 519	485 398
Lifetime special education costs	0	145 292	145 292
Lifetime residential care costs	0	179 856	192 254
Total costs to death	277 830	671 667	822 944

Table 3 *Outcome related to lifetime costs (£) discounted at 5%*

	1979	*1980*	*1981*
Cost per survivor	14 623	23 161	20 574
Cost per quality adjusted life	14 818	26 340	22 242
Cost per QALY	766	1 366	1 152

residential care. In 1980 and 1981 there was a higher incidence of disability, particularly severe (group 4) disability, which accounted for the considerably greater total costs to death.

The cost of initial hospital admission for each infant treated showed a relatively small variation over the three year study period. The progressively increasing total costs were incurred mainly as a result of the disabilities present in the survivors, but later hospital costs also rose sharply.

The measures of outcome are related to cost in table 3. Up to the age of 4 cost per survivor (cost of all infants divided by the number of survivors), cost per quality adjusted life, and cost per QALY declined in 1980 and 1981. This was a consequence of reduced mortality. The effect of the increased incidence of disability is seen in the behaviour of lifetime costs. They increased by all three outcome measures by amounts ranging between 41% and 78% between 1979 and 1980–81.

Discussion

The three year study covered a period during which there was considerable change in clinical practice in the regional neonatal intensive care unit after the appointment of a consultant neonatologist in January 1980. This is reflected in the outcome of the infants treated. There was a pronounced increase in the number of infants who survived, but also an increase in the number of disabled children. As the children were followed up to the age of 4, the increased cost of disability among the survivors became evident. The projection of costs from the age of 4 to death depends on assumptions of life expectancy, the degree of care that will be required, and the appropriate discount rate. Any variation in these assumptions will have a potentially profound effect on lifetime costs. A further area of concern in interpreting the results relates to random variation. Because the proportion of disabled survivors is small and because the disabled have a disproportionate effect on the cost of care, the confidence intervals of the cost estimates are wide.

The findings give an account of the work of a neonatal intensive care unit over a three year period and show the impact of continuing improvements in techniques. They do not, however, by themselves represent an evaluation of neonatal intensive care that would allow an estimate of the number of QALYs gained and the cost at which they were purchased. By no means all the QALYs that were produced can be described as gains attributable to neonatal intensive care because 35% of the cohort survived without ventilation. Furthermore, a full evaluation would entail comparison with a control group of similar infants treated by other means. A randomised controlled trial would be methodologically ideal but ethically unsupportable. Therefore researchers have attempted to construct control groups from infants treated before the advent of modern neonatal intensive care techniques.[5] The problems of retrospective pricing and clinical assessment make this the weak link in most 'before and after' studies, but even this technique with all its imperfections is increasingly difficult to apply. The dispersion of neonatal intensive care makes it unlikely that any control group can be found in the United Kingdom that is entirely unaffected by neonatal intensive care techniques.

One way of avoiding the difficulty of obtaining a control group is to assume that all those survivors who were ventilated would have died had they not been ventilated. This would be an unsafe assumption for cohorts of heavier infants but it is not unreasonable for infants from the very lowest birthweight ranges. We suppose that the 45 survivors of the 1980 and 1981 cohorts who were ventilated can be claimed as gains for neonatal intensive care. The 24 infants who survived without ventilation represent 35% of the population. This is roughly consistent with the proportion of VLBW infants that is known to have survived before the introduction of modern neonatal intensive care.[8] If neonatal intensive care can claim 65% of the survivors in 1980–1981, it was also responsible for nine of the 11 with disabilities, and a high proportion of the long term costs of care. Calculations on a sample restricted to infants who received ventilation gave the following approximate figures: cost per survivor £29 000; cost per quality adjusted life £33 000 and cost per quality adjusted life year £1770.

It will be for others to decide whether lives and life years gained at these prices represent good value for money or whether medical resources might be better used. A single study is not an adequate basis for any such decision. Neonatal intensive care is one of the most rapidly advancing specialties in medicine, so the balance between costs and benefits is almost certainly changing. Furthermore, neonatal intensive care is one of the few treatment regimens that has been systematically evaluated, at least in some centres. Similar studies do not exist for most common treatment regimens so it is not possible to compare the costs of neonatal intensive care with other important diagnostic groups.

It is also possible to question the validity of the concept of the QALY as a basis for comparing treatments. The cost per QALY gained is a measure of efficiency. It relates outputs to inputs and says nothing about what is fair or equitable. Even if it were agreed that medical resources should be allocated efficiently rather than equitably, problems still remain with the QALY as a measure of efficiency.

The QALY is intended as a measure for discriminating among different groups of patients. As such it is a bold attempt to weigh up the costs and the benefits of neonatal intensive care against, for example, heart transplantation, or hip replacement. It also offers possibilities for improving health service efficiency by comparing units that provide similar services. In our previous report on the cost of neonatal intensive care a comparison between two units was made.[7] This was not a methodologically sound comparison, but there was a strong suggestion that radically different clinical policies were being pursued. The cost of a QALY gained will differ between neonatal intensive care units as a result of differences in cost efficiency and clinical policy. Comparisons of the cost per QALY among different units could be seen as a means of disseminating best practices more widely or, alternatively as a threat to clinical independence.

QALYs are intended to provide a systematic measure for discriminating among different diagnostic groups but many will object because they depend on the placing of subjective values on human disability. There is no sound reason for deciding whose opinion should be considered—for example, parents, clinicians, tax payers, or politicians. Boyle *et al* asked the opinions of parents who expressed the view that a negative value should be attached to some severely disabled lives.[5] This would be anathema to many who would include the failure to consult the patient in their list of objections.

Although it is only recently that QALYs have been used in the evaluation of health care, the ethical concepts underlying them have been debated for a considerable time.[9] [10] QALYs are an example of the consequentialist approach that justifies actions in terms of the greatest good for the greatest number or, in the context of this study, the maximum output for a given input. Utility is presumed to be derived only from outcomes and not from the process by which these outcomes are achieved. In many areas of medicine, especially those in which outcomes are predictable, the processes by which they are reached are of considerable importance. This is plain enough in the treatment of terminal illness. It also applies to neonatal intensive care units, where a measure of efficiency based solely on medical outcomes may understate the value of its outputs.

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References

[1] Mutch LMM, Brown NJ, Speidel BD, Dunn PM. Perinatal mortality and neonatal survival in Avon: 1976–9. *Br Med J* 1981;**282**:119–22.

[2] Lee K-S, Paneth N, Gartner LM, Pearlman MC, Gruss L. Neonatal mortality: an analysis of the recent improvement in the United States. *Am J Public Health* 1980;**70**:15–21.

[3] Pharoah POD. Childhood epidemiology: perspectives and patterns. *Br Med Bull* 1986;**42**:119–26.

[4] Williams A. Economics of coronary artery bypass grafting. *Br Med J* 1985;**291**:326–9.

[5] Boyle MH, Torrance GW, Sinclair JC, Horwood SP. Economic evaluation of neonatal intensive care of very low birthweight infants. *N Engl J Med* 1983;**308**:1330–7.

[6] Powell TG, Pharoah POD, Cooke RWI. Survival and morbidity in a geographically defined population of low birthweight infants. *Lancet* 1986;**i**:539–42.

[7] Sandhu B, Stevenson RC, Cooke RWI, Pharoah POD. Cost of neonatal intensive care for very low birthweight infants. *Lancet* 1986;**i**:600–3.

[8] Pharoah POD, Alberman ED. Mortality of low birthweight infants in England and Wales 1953 to 1979. *Arch Dis Child* 1981;**56**:86–9.

[9] Gillon R. Philosophical medical ethics. Deontological foundations for medical ethics. *Br Med J* 1985;**290**:1331–3.

[10] Gillon R. Philosophical medical ethics. Utilitarianism. *Br Med J* 1985;**290**:1411–3.

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Dilemmas associated with antenatally detected urinary tract abnormalities

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SUMMARY Over a five year period 55 fetuses had abnormalities of the urinary tract detected by antenatal ultrasound scan. The incidence was 1:935 total births during a one year prospective study. Intrauterine intervention was undertaken in five for suspected obstructive uropathy, which was confirmed in only two. Of 51 live born infants, five died (two with renal failure), and only 18 (35%) had a clinically detectable abnormality at birth. Twenty seven patients underwent postnatal operations, the remainder being treated conservatively.

Antenatal counselling was seldom undertaken by those responsible for the postnatal care. There were many instances of prospective parents receiving little or inappropriate information. Greater cooperation is required between all the staff concerned particularly as the natural history and appropriate postnatal management of some urinary tract abnormalities are still not known.

The use of real time ultrasonography in obstetric practice has resulted in an increase in the detection rate of fetal urinary tract abnormalities in district hospitals as well as in specialist centres.[1 2] Any suggestion of fetal abnormality on the antenatal scan can cause great anxiety and distress to the parents.[3] Abnormalities can, however, be difficult to characterise fully given the limitations of ultrasonography and the relative paucity of knowledge about the natural history of developmental lesions.[4] Previous reports have documented a poor correlation between the antenatal findings and the postnatal diagnoses.[5 6]

Problems of diagnostic accuracy are combined with uncertainty about both the prognosis of many of the abnormalities and of the role of prenatal surgery.[7 8] Urinary tract abnormalities occur in about 1:1200 pregnancies[9] and hence the individual members of the obstetric and paediatric staff are likely to have only limited experience with these lesions. Poor communication among staff in different specialities will only add to the difficulties of counselling the parents both antenatally and postnatally. We report our experience to illustrate some of the diagnostic and therapeutic dilemmas that arise when urinary tract abnormalities are detected antenatally. We emphasise the need for good communication and exchange of information among staff in the departments of obstetrics, radiology, and paediatrics.

Patients and methods

Between January 1982 and December 1986, 55 cases of antenatally diagnosed urinary tract abnormalities came to our attention either by referral for postnatal management, notification by the perinatal pathologist, or (latterly) referral for antenatal counselling. The number of cases increased from five in 1982 to 21 in 1986. The data were collected prospectively during 1986 and included fetal urinary tract abnormalities that resulted in termination of pregnancy or intrauterine fetal death.

It is the policy in all the referring centres to perform a routine ultrasound scan between 16 and 20 weeks' gestation. Further ultrasound examinations were for obstetric reasons except in one Nottingham centre where serial ultrasound scans were routinely carried out between 28 and 34 weeks as part of a research project into fetal growth. The ultrasound scans were performed by trained radiographers who called in a radiologist for more detailed scanning if an abnormality was detected or strongly suspected—for example, if oligohydramnios was seen.

Five mothers were referred to a specialist centre where antenatal bladder drainage procedures were performed. These comprised insertion of an intrauterine vesicoamniotic shunt in three fetuses, and bladder aspiration in two (table 1).

Results

The gestational age of detection of the urinary tract abnormality ranged from 18 to 39 weeks with a mean of 32 weeks (mode 32 weeks). Forty one of the 55 fetuses were male and 14 female. In most instances the antenatal scan had been reported as showing hydronephrosis or cystic areas in the kidney(s), with few specific diagnoses. Infantile polycystic kidneys had been suggested inappropriately on two occasions.

During a prospective study of the 10 291 births in Nottingham in 1986, 11 urinary tract abnormalities were found, an incidence of 1·07 per thousand. Three pregnancies were reported as showing mild hydronephrosis in one or both fetal kidneys on scans late in pregnancy but these were not confirmed on subsequent antenatal or postnatal scans.

Five fetuses were thought to have obstructive uropathy (posterior urethral valves) from the ultrasound findings, and underwent intrauterine operation (table 1). Only two of the five were subsequently proved to have urethral valves at necropsy and both died before 22 weeks' gestation. The other three infants had non-obstructive uropathy with megalocystis megaloureter in two (one with prune belly syndrome), and bilateral vesicoureteric reflux in the other. All three are alive but one has chronic renal failure due to associated dysplastic kidneys.

Of the 55 fetuses, 51 were born alive. In addition to the two fetuses with posterior urethral valves (table 1), one pregnancy was terminated at 19 weeks because of oligohydramnios (the kidneys were normal at necropsy), and one fetus was stillborn at 34 weeks' gestation with Potter's syndrome.

Five of the 51 infants born alive subsequently died (table 2). Two died of renal failure at 4 hours and 14 days, with bilateral multicystic kidneys and posterior urethral valves, respectively. One infant died at 3 days of a pulmonary embolus unrelated to the renal problem of bilateral obstruction of the pelviureteric junctions. One died of a hypoplastic left heart, and one of a ventricular septal defect together with trisomy 18 syndrome and a persistent urachus.

Table 1 *Details of intrauterine operations in five patients*

Gestational age at detection (weeks)	*Gestational age at operation (weeks)*	*Intrauterine operation*	*Postnatal diagnosis*	*Outcome*
19	20	Vesicoamniotic shunt	Posterior urethral values	Termination of pregnancy at 21 weeks' gestation Deletion long arm chromosome 2
20	20	Vesicoamniotic shunt	Megalocystis megaloureters*	Alive; chronic renal failure
20	21	Vesicoamniotic shunt	Posterior urethral valves	Intrauterine fetal death at 22 weeks' gestation
18	20, 30, 31	Bladder drainage by aspiration	Megalocystis, megaloureters	Alive and well
33	33	Bladder drainage by aspiration	Bilateral vesicoureteric reflux (left worse than right)	Alive and well

*Prune belly syndrome.

Table 2 *Diagnosis and outcome in 51 infants born alive*

Diagnosis	*No of patients*	*No with bilateral disease*	*No of operations*	*No of deaths*
Obstruction of the pelviureteric junction	25	6	19	1
Multicystic dysplastic kidney	10	1	2	2
Vesicoureteric reflux	4	2	2	0
Megalocystis megaloureter	3	0	0	0
Posterior urethral valves	2	0	1	1
Obstruction of the vesicoureteric junction	2	0	2	0
Prune belly syndrome	1	0	0	0
Persistent urachus	1	0	0	1
Duplex kidney with dysplasia	1	0	1	0
Solitary normal kidney	1	0	0	0
Two normal kidneys	1	0	0	0

There were two other infants who also had chromosomal abnormalities. The infant with Turner's syndrome and obstruction to the left pelviureteric junction survived, but termination of pregnancy was carried out for the fetus with deletion of the long arm of chromosome 2 and posterior urethral valves.

Only 18 (35%) of the problems were clinically apparent at birth. All patients were initially investigated by ultrasonography in the neonatal period, and subsequently 34 (67%) had micturating cystourethrograms; 25 (49%) ^{99m}technetium diethylenetriamine penta-acetic acid renal scans; 20 (39%) intravenous urograms and six (12%) ^{99m}technetium dimercaptosuccinic acid scans.

Of the 51 infants born alive 27 had been operated on at the time of writing. Eighteen patients had had pyeloplasties; four had reimplantation of the ureters, four had nephrectomies (one heminephrectomy), and one patient had diathermy of the posterior urethral valves. The remaining 24 are being managed conservatively and some may require operation later.

Discussion

The present series documents an incidence of antenatally detectable urinary tract abnormalities of 1:935 pregnancies. This compares with the 1:1200 incidence quoted by Kullendorff *et al* in a 24 month prospective screening programme in Lund,[9] and the incidence of approximately 1:333 reported from Malmo.[10] Although the latter study detected 33 abnormalities in 11 986 pregnancies over five years with routine scanning at 17 and 33 weeks' gestation there were 10 cases of hydroureter, hydronephrosis, or solitary cyst that extensive postnatal investigation showed to be only mild dilatation of the ureters or renal pelvis, or both, with no definitive diagnosis.

During our prospective study in 1986 three instances of transient dilatation of the fetal renal pelvis were noted. Such transient dilation is not uncommon in the first half of pregnancy but its recognition later on does raise the dilemma of how far to investigate postnatally if the follow up ultrasound scan is normal. Smith *et al* recognised two infants with mild hydronephrosis in utero who had normal postnatal scans and were discharged from follow up. Both were subsequently shown to have vesicoureteric reflux when investigated later, one for urinary tract infection and one for septicaemia.[1]

The diagnostic accuracy of the antenatal ultrasound scans did seem to improve during the study and this probably reflects improvement in the technical field as well as the learning curve of the ultrasound operators. Though Pocock *et al* suggested that the antenatal ultrasound examination correctly diagnosed the urological abnormality in all but four of 28 patients, the ultrasound diagnosis was often non-specific, as in our series, and consisted largely of hydronephrosis or cystic appearances in both kidneys.[11] Of some concern are the instances of incorrect diagnoses being made—for example, infantile polycystic kidneys. The prognosis for this condition may be poor, and one set of parents in our series were counselled inappropriately because the fetus was subsequently shown to have apparently normal kidneys. Another infant had severe bilateral vesicoureteric reflux.

Because only 35% of urinary tract abnormalities were clinically detectable at birth there have been many management dilemmas posed by the detection of problems that might have remained clinically silent. The need for removal of an asymptomatic multicystic dysplastic kidney and the timing of operation for obstruction to the pelviureteric junction are two such examples.[12 13]

More important is the place of antenatal surgical intervention.[8 14 15] The fetus with a unilateral renal lesion can safely be observed if a normal kidney is present on the other side and there is no evidence of oligohydramnios. The dilemma arises if the fetus has both kidneys affected. In a highly selected series of 73 placements of catheter shunts for fetal obstructive uropathy reported to the International Fetal Surgery Registry, the death rate associated with the operation was 4·6%.[16] The procedure was undertaken at a mean (SD) of 24·2 (5) weeks (range 17 to 36) and 30 fetuses (41%) survived. Twenty seven of the 29 postnatal deaths were due to pulmonary hypoplasia, and of the 30 surviving fetuses, 16 had posterior urethral valves, one had urethral atresia, three had prune belly syndrome, and 10 died of unknown causes. The reasons for selection and lack of details about the patients makes interpretation of any possible benefit difficult.

Our own series highlights the problem of differentiating true obstructive uropathy from nonobstructive lesions, and we further question the role of untrauterine intervention. The fetus with bilateral renal disease associated with oligohydramnios early in pregnancy seems to have a poor prognosis with a high incidence of chromosomal abnormalities.[2] Most urinary tract abnormalities, however, will be recognised on a scan later in the pregnancy. In the rare instance of a fetus developing increasing bilateral hydronephrosis from suspected obstructive uropathy, early delivery in an appropriate neonatal unit may be preferable to antenatal operation.

Reviewing our experience has led us to assess critically the lines of communication that are used when an abnormality is detected antenatally. A report of such an abnormality by the radiographer to the referring obstetrician may result in a request to a radiologist for a repeat scan, referral of the patient to a specialist obstetrics centre, enlistment of the help of a paediatric physician or surgeon, or a 'wait and see' policy. Counselling is generally done by the obstetricians. Though we do not have accurate information about the timing or content of parental counselling we do know of many instances of inappropriate counselling, and also of parents not being told of abnormalities before the birth of their child. In most instances the paediatric staff were not informed before the birth of the child either, and this has also been reported from other centres. It is obvious that there should be greater interchange of information among staff in the departments of obstetrics, radiology, paediatrics, and clinical genetics. All parents that we have spoken to have suggested that more information would have been welcome and the sharing of knowledge among the disciplines can only help us all to traverse the learning curve. Discussion of our own local experience with our obstetric colleagues has led to more requests for antenatal counselling by the paediatric staff.

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References

[1] Smith D, Egginton JA, Brookfield DSK. Detection of abnormality of fetal urinary tract as a predictor of renal tract disease. *Br Med J* 1987;**294**:27–8.

[2] Nicolaides KH, Rodeck CH, Gosden CM. Rapid karyotyping in non-lethal fetal malformations. *Lancet* 1986;i:284–7.

[3] Furness ME. Reporting obstetric ultrasound. *Lancet* 1987; i:675–6.

[4] Thomas DFM. Urological diagnosis in utero. *Arch Dis Child* 1984;**59**:913–5.

[5] Turnock RR, Shawis R. Management of fetal urinary tract anomalies detected by prenatal ultrasonography. *Arch Dis Child* 1984;**59**:962–5.

[6] Avni EF, Rodesch F, Schulman CC. Fetal uropathies: diagnostic pitfalls and management. *J Urol* 1985;**134**:921–5.

[7] Broyer M, Guest G, Lestage F, Gacoin FE. Prenatal diagnosis of urinary tract malformations. *Adv Nephrol* 1985;**14**:21–38.

[8] Nicolaides Kh, Rodeck CH. Fetal therapy. In: Strudd J, ed. *Progress in obstetrics and gynaecology 5.* Edinburgh: Churchill Livingstone, 1985:36–57.

[9] Kullendorff CM, Larsson LT, Jorgensen C. The advantage of antenatal diagnosis of intestinal and urinary tract malformations. *Br J Obstet Gynaecol* 1984;**91**:144–7.

[10] Helin I, Persson P-H. Prenatal diagnosis of urinary tract abnormalities by ultrasound. *Pediatrics* 1986;**78**:879–83.

[11] Pocock RD, Witcombe JB, Andrews HS, Berry PJ, Frank JD. The outcome of antenatally diagnosed urological abnormalities. *Br J Urol* 1985;**57**:788–92.

[12] Maizels M, Wacksman J, Belman AB, Colodny AH. Management of multicystic dysplastic kidney. *Dialogues in Pediatric Urology* 1987;**10**:1–8.

[13] Homsy YL, Williot P, Danais S. Transitional neonatal hydronephrosis: Fact of fantasy? *J Urol* 1986;**136**:339–41.

[14] Kramer SA. Current status of fetal intervention for congenital hydronephrosis. *J Urol* 1983;**130**:641–6.

[15] Glick PL, Harrison MR, Adzick NS, Noall RA, Villa RL. Correction of congenital hydronephrosis in utero IV: in utero decompression prevents renal dysplasia. *J Pediatr Surg* 1984;**19**: 649–57.

[16] Manning FA, Harrison MR, Rodeck C. Catheter shunts for fetal hydronephrosis and hydrocephalus. Report of the International Fetal Surgery Registry *N Engl J Med* 1986;**315**:336–40.

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Pulmonary agenesis as part of the VACTERL sequence

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SUMMARY Pulmonary agenesis is an uncommon anomaly that has been reported in isolation and in association with other congenital defects. Such defects include oesophageal atresia, cardiac malformation, horseshoe kidney, and anal atresia. Over a period of three years we have seen five neonates or fetuses with unilateral agenesis of the lung. All the cases had three or more anomalies seen in the VACTERL sequence in addition to the pulmonary atresia. None had a tracheo-oesophageal fistula. None were the products of consanguineous marriages. There had been no recurrence of this range of defects in any of the familes at the time of writing. We suggest that pulmonary agenesis may occur as an alternative to tracheo-oesophageal fistula in the VACTERL sequence.

VACTERL is an acronym widely applied to a group of Vertebral, Anal, and Cardiac defects, Tracheo-Esophageal fistula, and Renal and Limb defects occurring in a non-random association.[1] Other acronyms have included VATER,[2] VACTEL, ARTICLE, TREACLE, and LEATHER, and in the last two the second E refers to 'Etcetera'.[3] Though the existence of an association is not in doubt, the broadest form of the sequence merges with caudal regression and complexes associated with choanal atresia (CHARGE) and Mullerian duct aplasia (MURCS). It may also represent the phenotype of several chromosome anomalies including trisomy 13 and 13q-. The designation 'syndrome' does not, therefore, seem appropriate.

Czeizel and Ludanyi,[4] in a comprehensive review, divided their cases into those with 'true' VACTERL in which three or more features were found alone, and 'mixed' VACTERL, in which there were other congenital anomalies. They also divided the features of the association into 'close' and 'broad' definitions (table 1). Using these stringent definitions true VACTERL becomes a more specific entity, which is reasonably well separated from similar phenotypes. The precise developmental mechanism has not been identified but all the defects can be explained by an

Table 1 *Definitions of VACTERL*[4]

Type of anomaly	*Close definition*	*Broad definition*
Vertebral anomalies	Absence of vertebra fusion Hemivertebra Spina bifida occulta	Other vertebral anomalies or rib absence or fusion
Anal atresia	With or without rectovaginal or perineal fistula including anal stenosis	Atresia with other gastrointestinal anomalies
Cardiac defects	Ventricular septal defect Atrial septal defect Tetralogy of Fallot	Other cardiovascular anomalies
Tracheo-**E**sophageal fistula	Includes atresia without fistula, and fistula without atresia	Oesophageal stenosis
Renal anomalies	Unilateral or bilateral renal agenesis or dysplasia	Accessory, double or fused kidneys, and others
Limb anomalies	Preaxial reduction (radial or ulnar) Preaxial polydactyly	Other reductions Other polydactylies

insult occurring during early embryogenesis. The radial defects have been explained as vascular abnormalities—for example, absence of the radial artery.[5]

Because the bifurcation of the tracheal primordium and the subsequent initiation of lung development occur within the period encompassed by the VACTERL sequence, it would not be surprising to find bilateral or unilateral pulmonary agenesis as part of the same range of defects.

Case reports

CASE 1

A female infant was born to a 26 year old primigravida after a prolonged labour requiring rotation with Kielland's forceps. The pregnancy had been uneventful except that the mother had had wisdom teeth removed during the first trimester. This had required heavy analgesia, which made her extremely ill; the identity of the drug was not known. At delivery the infant weighed 3140 g and had skeletal abnormalities. The right lung was thought to be collapsed and the infant died despite intensive care. At necropsy there was right pulmonary agenesis as well as other anomalies (table 2).

CASE 2

A female infant died after three days with poor respiratory function, radial aplasia, aplasia of the left pectoralis major, and a 'hypoplastic' left lung (fig 1). She had been delivered after spontaneous premature labour at 36 weeks' gestation. Severe intrauterine growth retardation had been noted at 18 weeks. At necropsy left pulmonary agenesis together with skeletal, urogenital, and anal anomalies were found (table 2). Two previous pregnancies had been uneventful and had resulted in babies born alive.

CASE 3

A stillborn male infant with multiple anomalies was delivered electively at 31 weeks' gestation after ultrasound investigation of the 23 year old primigravida mother had shown oligohydramnios and renal agenesis. (table 2).

CASE 4

The mother of this infant was a 29 year old woman with two live children. Spontaneous onset of labour was followed by emergency lower segment

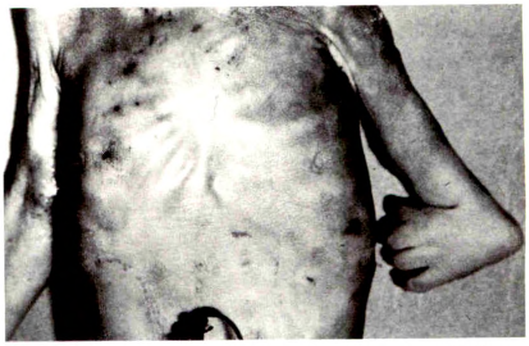

Fig 1 *Absence of pectoral muscles, shortening of left arm, and reduction anomalies of forearm and hand (case 2).*

Table 2 *VACTERL anomalies in this series; broad features in parentheses*

Anomaly	*Case 1*	*Case 2*	*Case 3*	*Case 4*	*Case 5*
Vertebral	No (13 ribs)	Cervical hemivertebrae	No *x*-ray	Cervical hemivertebrae	Lumbosacral and thoracic hemivertebrae
Anal	No	No	Anal atresia	Anal atresia	Anal atresia
Cardiac	Atrial septal defect	No	Atrial and ventricular septal defects	Atrial and ventricular septal defects (Aortic stenosis)	Atrial and ventricular septal defects
T-E fistula	(Agenesis right lung)	(Agenesis left lung)	(Agenesis right lung)	(Agenesis left lung)	(Agenesis left lung)
Renal	No (Genital anomalies)	No	Bilateral renal dysplasia	No (Horseshoe kidney)	No (Horseshoe kidney and genital anomalies)
Limb	Bilateral radial aplasia and absent right thumb	Left radial aplasia, thumb hypoplasia, and absent pectorals	No *x*-ray	Bilateral radial aplasia and proximal humoral aplasia; absent digits	Left radial aplasia and absent digits; left femoral hypoplasia
Total No of close anomalies	2	2	3	4	4
Total No of close and broad anomalies	(5)	(3)	(4)	(6)	(6)
Total No of close anomalies if lung agenesis is included	3	3	4	5	5

caesarean section for fetal distress. A malformed female infant was delivered and the Apgar scores were poor (fig 2). Medical support was discontinued after two hours. The abnormalities found at necropsy are listed in table 2.

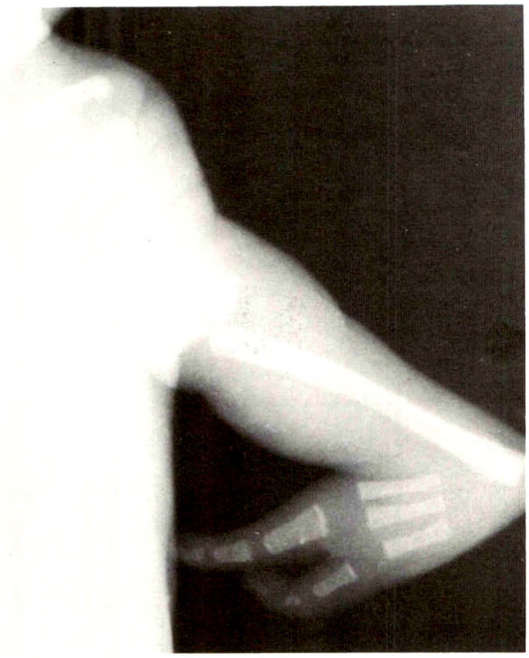

Fig 2 *X-ray showing radial aplasia, absent thumb, and macrosyndactyly (case 4).*

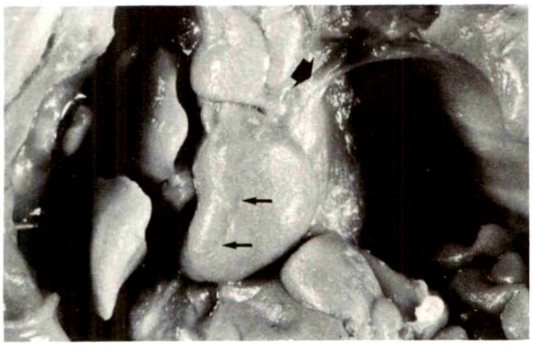

Fig 3 *Thoracic contents showing absence of left lung and hemidiaphragm, persistent left superior vena cava (broad arrow) and abnormal cardiac outline with hypoplastic right ventricle. Small arrows indicate anterior descending coronary artery. Loops of intestine protrude into left hemithorax, and right lung is relatively well developed (case 5).*

CASE 5

A fetus was referred for histological examination as a missed and macerated abortion. In addition to the anomalies listed in table 2, examination showed a sacral skin tag, a hypoplastic left lower limb, a large left diaphragmatic hernia, 13 pairs of ribs, and a persistent left superior vena cava (fig 3). The gestational age was estimated at 14–15 weeks at the time of death, no cause for which was found.

Discussion

Pulmonary agenesis has been classified morphologically by the extent to which bronchopulmonary tissue is absent. Earlier classifications were modified by Spencer[6] who divided pulmonary agenesis into: (i) bilateral complete agenesis; (ii) unilateral agenesis with (a) complete absence of bronchi, alveolar tissue, and blood supply, or (b) rudimentary bronchus present but not invested by pulmonary tissue, or (c) poorly developed main bronchus with fleshy mass and poorly organised parenchyma; and (iii) lobar agenesis or lesser forms of congenital anomaly.

Variation in lobation or segmentation in the human lungs is extremely common, and usually of practical importance only to those surgeons who operate on the intrathoracic organs. The complete absence of a lung together with its bronchus is, on the other hand, sufficiently rare to excite considerable interest. The defect commonly occurs in association with other congenital anomalies. Schechter reviewed published reports on the 'congenital subtractive bronchopneumonic malformations'.[7] In 114 cases of complete absence of one or both lungs, other anomalies were detected in the skeleton (n=30), the great vessels (n=27), the cardiac anatomy (n=26), the urogenital system (n=15), the upper respiratory tract (n=13), the face and cranial nerves (n=13), the lower intestinal tract (n=8), and the oesophagus (n=7).

The absence of one lung in association with preaxial reduction anomalies of the upper limb was first described in 1974 as an extreme expression of Poland's syndrome.[8] In this case the ipsilateral diaphragm was also absent, and there were multiple rib anomalies but no defects of the heart or anus. A further report described triphalangeal thumb, rib anomalies, and a transient cardiac murmur in association with left pulmonary agenesis.[9] A more recent report of four cases from Saudi Arabia described cases of unilateral lobar or lung aplasia associated with other anomalies.[10] Three had preaxial upper limb anomalies including triphalangeal thumb, duplicated thumb, and posteriorly placed and contracted thumb. Other individual defects included rib fusion, spina bifida, and flexion contractures. All four cases had atrial septal defects, and all four were the products of consanguineous marriages.

All our cases come into Spencer's category (iia), having no evidence of rudimentary bronchi though case 4 had an area of dysplastic right lung parenchyma that could conceivably represent an intrapulmonary sequestrated remnant of the left lung. On histological examination the area resembled cystic adenomatoid malformation and, because there was no evidence of a left main bronchus, the latter explanation therefore appears more probable. Table 2 shows that all five cases had several features of the VACTERL sequence and, if tracheo-oesophageal fistula is replaced by pulmonary agenesis, all can be defined as having the 'close' VACTERL sequence. Over the period that these cases presented we saw no other cases of pulmonary aplasia. We suggest therefore that there is a genuine association between pulmonary agenesis and the other features of the VACTERL sequence, and that the risk of recurrence in subsequent pregnancies is likely to be low.

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References

[1] Kaufman RL. Birth defects and oral contraceptives. *Lancet* 1973;**i:**1396.

[2] Quan L, Smith DW. The VATER association: Vertebral defects, Anal atresia T-E fistula with esophageal atresia, Radial and Renal dysplasia. A spectrum of associated defects. *J Pediatr* 1973;**82:**104–7.

[3] Landing BH. Syndromes of congenital heart disease with traceobronchial anomalies. *AJR* 1975;**123:**679–86.

[4] Czeizel A, Ludanyi I. VACTERL-Association. *Acta Morphol Hung* 1984;**32:**75–96.

[5] Hoyme HE, VanAllen MI, Lyon Jones K. The vascular pathogenesis of some sporadically occurring limb defects. *Sem Perinatol* 1983;**7:**299–306.

[6] Spencer H. *Pathology of the lung.* 3rd ed. Oxford: Pergamon Press, 1977:71–114.

[7] Schechter DC. Congenital absence or deficiency of lung tissue. The congenital subtractive bronchopneumonic malformations. *Ann Thorac Surg* 1968;**6:**286–313.

[8] Frias JL, Felman AH. Absence of the pectoralis major with ipsilateral aplasia of the radius, thumb, hemidiaphragm and lung: an extreme expression of Poland's anomaly? *Birth Defects* 1974;**10:**55–9.

[9] Manouvrier S. Aplasia pulmonaire et triphalange du pouce: une nouvelle association malformative? *Larc Medical* 1983;**iii:**373–5.

[10] Mardini MK, Nyhan WL. Agenesis of the lung. Report of four patients with unusual anomalies. *Chest* 1985;**87:**522–7.

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Lung function and bronchial responsiveness measured by forced oscillometry after bronchopulmonary dysplasia

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SUMMARY Forced oscillometry was used to investigate whether lung injury due to bronchopulmonary dysplasia in eary life caused abnormalities in bronchial calibre or an increase in bronchial responsiveness to histamine at school age. Results were compared with data obtained from healthy children born prematurely and from healthy children born at term. There was a mild increase in frequency dependence of total respiratory resistance in children who suffered from lung injury in early life, which indicates uneven ventilation in peripheral airways. Bronchial responsiveness to histamine in these subjects was normal. No abnormalities were found in the control group.

We conclude that lung injury in early life may cause residual abnormalities of peripheral airways. This does not happen in premature babies who do not have respiratory problems in the neonatal period.

Pulmonary injury during the first years of life when the lungs are growing rapidly,[1 2] can induce bronchiolar lesions that may interfere with alveolar and vascular growth.[3 4] Some investigators have found a high incidence of long term pulmonary sequelae in premature babies with idiopathic respiratory distress syndrome,[5–8] whereas others have found few residual pulmonary abnormalities.[9–15]

In this study we looked for indicators of long term airway damage—that is, abnormal respiratory resistance and frequency dependence of resistance—and for bronchial hyper-responsiveness to histamine in 15 children who had idiopathic respiratory distress syndrome with bronchopulmonary dysplasia as neonates, and in 15 children who were born prematurely but did not have idiopathic respiratory distress syndrome.

Subjects and methods

We investigated two groups. Group 1 comprised 15 children aged 3·3 to 10·6 years (mean 6·3) who were born prematurely at gestational ages of 26 to 34 weeks (mean 28·1) and had idiopathic respiratory distress syndrome. They were mechanically ventilated with moderate to high oxygen mixtures for one to 46 days (mean 21·9 days) and developed severe bronchopulmonary dysplasia diagnosed by radiological[16] and clinical[17] criteria (table 1). Group 2 comprised 15 children aged 3·8 to 8·0 years (mean 5·9) who were born at gestational ages of 30 to 35 weeks (mean 31·9). None had important respiratory symptoms during the neonatal period or in later life (table 2).

According to their parents none of the children had upper or lower respiratory tract symptoms during the two weeks before the study. A history of current and past respiratory symptoms (for example, recurrent episodes of cough and wheezing) and a family history of asthma or hay fever in first and second degree relatives was taken by an interviewer (ED) who completed a questionnaire.[18]

As many children in this study were less than 5 years of age lung function was measured by the forced oscillation technique.[19] A pseudorandom noise pressure signal containing all harmonics of 2–26 Hz is applied to the mouth with a loudspeaker. Mouth pressure and flow signals recorded by two identical differential transducers (Validine MP445) are fed into a Fourier analyser, which averages these values over a time interval of 16 seconds and calculates the impedance of the respiratory system at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, and 26 Hz. Forced oscillation is a convenient method for children from about 2½ years of age onwards because only passive cooperation is needed. During measurements the child is seated and the apparatus is connected with a mouth piece. The nose is clipped and the floor of the mouth and cheeks are supported to eliminate shunting of airflow to the upper airways. With forced oscillation total respiratory resistance (R_{rs}) in cm H_2O/l/second and reactance are measured simultaneously over the frequency range of 2–26 Hz. R_{rs} is determined mainly by the patency of the central airways, and is dependent to only a limited degree on the resistance of the lungs and chest wall.[20] Because R_{rs} is measured over a frequency spectrum, the frequency dependence of R_{rs} (dR_{rs}/df) in cm H_2O/l/second[2] can also be calculated.[21 22] dR_{rs}/df is a measure of unevenness of ventilation, and is determined by the patency of the peripheral airways. Using the forced oscillation technique, we selected the resistance of the respiratory system at an oscillation frequency of 6 Hz (R_{rs6}) as a measure of the patency of the central airways, and dR_{rs}/df as a measure of the calibre of the peripheral airways. R_{rs6} was chosen because an oscillation frequency of 6 Hz was the lowest frequency with a favourable signal:noise ratio.[20] All children had a physical examination before the measurements.

Bronchial responsiveness was measured by a histamine challenge test. Histamine was administered through a Rosenthal-French dosimeter connected to a DeVilbiss nebuliser (type 646). The dosimeter was operated by compressed air at 20 psi with a timing adjustment of 0·6 seconds. Doses of histamine biphosphate in saline of 2·5, 5, 10, 20, 40, 80, 160, and 320 μg were given, each in four inhalations of 5 μl at five minute intervals. Saline was inhaled before histamine to exclude non-specific responses. Each challenge was preceded by five consecutive forced oscillation measurements from which the mean baseline value was calculated. The mean of three technically satisfactory forced oscillation measurements was used to construct a dose response curve. $PD_{40}R_{rs6}$, defined as the dose of histamine that caused a 40% increase from the mean baseline R_{rs6}, was calculated from the dose response curve by linear interpolation of data points. We have previously shown that $PD_{40}R_{rs6}$ is closely correlated with the dose of histamine that caused a 20% increase in forced expiratory volume in one second ($PD_{20}FEV_1$).[23] Bronchial challenge was stopped after inhalation of 320 μg histamine or after $PD_{40}R_{rs6}$ had been obtained.

Mean predicted baseline values of R_{rs6} and dR_{rs}/df were taken from a reference population with no past or current history of pulmonary injury, asthma, or other recurrent respiratory disease.[19] Forced oscillation and bronchial responsiveness values were expressed as standard scores, calculated from the formula:mean measured value − mean predicted value ÷ by the standard deviation of the mean predicted value. Baseline R_{rs6} was considered increased if it was higher than +1·65 standard scores and dR_{rs}/df decreased if it was lower than −1·65 standard scores (95% confidence intervals from the predicted mean). Bronchial responsiveness was considered increased if $PD_{40}R_{rs6}$ was lower than −1·65 standard scores from bronchial responsiveness measured by forced oscillation in a reference population of healthy children born at term aged 5–8 years (that is reacting to less than 300 μg histamine).[24]

For ethical reasons we were not allowed to perform skin prick tests or sample blood gases but written informed consent was obtained from the parents. Wilcoxon's two sample test was used to analyse the significance of differences between the groups for baseline values and $PD_{40}R_{rs6}$ histamine. A p value of $<0·05$ was considered significant.

Results

Six of the children who had bronchopulmonary dysplasia had persistent symptoms at the time of investigation; one (case 6) had symptoms induced by exercise. Two had symptoms that disappeared after the age of 4 (cases 7 and 8). Seven children never had symptoms after their discharge from hospital (table 1). There was no difference in the mean age at the time of investigation between children with and without symptoms. None of the children who had not had idiopathic respiratory distress syndrome had a history of past or current respiratory symptoms (table 2). Three children who had bronchopulmonary dysplasia (20%) and three children who had not had idiopathic respiratory distress syndrome (20%) had a first or second degree relative with asthma or hay fever.

Of the six children who had bronchopulmonary dysplasia with current symptoms R_{rs6} was increased in three. Decreased dR_{rs}/df values were found in four. Of the nine children without current symptoms, R_{rs6} was increased in one and dR_{rs}/df was decreased in two (figure, table 1). In all children who had not had idiopathic respiratory distress syndrome, R_{rs6} values were within the normal range. dR_{rs}/df was decreased in one (figure, table 2).

Table 1 *Children who had had bronchopulmonary dysplasia*

Case No	*Age at investigation (years)*	*First or second degree relative with asthma*	*Gestational age (weeks)*	*Scores:*		*Standard scores*		$PD_{40}R_{rs6}$	
				Northway et al[16]	*Toce et al*[17]	R_{rs6}	$\overline{dR_{rs}/df}$	*Dose of histamine (μg)*	*Standard score*
With current respiratory symptoms:									
1	4·7	No	32	3	23	2·13	−2·07	>320	
2	6·1	No	29	4	20	−0·64	−1·17	100	−3·58
3	5·0	No	28	4	20	0·96	−0·50	>320	
4	6·8	No	29	4	20	0·87	−3·50	>320	
5	4·4	No	27	3	20	1·84	−4·00	>320	
6	7·0	Yes	32	3	10	1·74	−3·50	>320	
With past, but not current respiratory symptoms:									
7	6·8	No	28	3	22	0·75	−1·50	>320	
8	9·2	Yes	29	4	25	0·28	−1·50	290	−1·76
With no respiratory symptoms:									
9	6·2	No	34	3	12	1·19	−2·17	180	−2·56
10	5·5	No	32	3	19	0·31	−1·50	>320	
11	10·0	Yes	32	3	3	−0·28	0·67	>320	
12	3·3	No	28	3	15	1·11	−0·33	240	−2·08
13	7·2	No	29	2	20	−0·19	−0·67	>320	
14	5·9	No	26	3	5	1·96	−1·33	>320	
15	4·4	No	28	1	10	0·16	−1·83	>320	

Table 2 *Children born prematurely without idiopathic respiratory distress syndrome*

Case No	*Age at investigation (years)*	*First or second degree relative with asthma*	*Gestational age (weeks)*	*Standard scores*		$PD_{40}R_{rs6}$	
				R_{rs6}	$\overline{dR_{rs}/df}$	*Dose of histamine (μg)*	*Standard scores*
16	7·3	No	35	0·80	−0·23	>320	
17	8·0	No	34	0·92	−0·83	>320	
18	7·5	No	32	−2·55	2·76	220	−2·22
19	4·2	No	32	0·82	3·63	>320	
20	6·8	Yes	31	−1·01	1·36	220	−2·22
21	6·8	Yes	31	−1·16	0·88	>320	
22	5·5	No	32	0·02	0·16	>320	
23	5·4	No	32	0·19	0·50	>320	
24	6·7	No	32	0·19	0·50	>320	
25	6·6	No	31	1·09	3·18	>320	
26	3·8	No	32	0·10	0·07	200	−2·38
27	4·3	No	31	−0·95	4·17	>320	
28	3·8	No	31	−1·22	3·33	>320	
29	7·0	No	30	−1·85	3·83	>320	
30	4·3	Yes	33	0·98	−2·17	>320	

The mean R_{rs6} was significantly higher in the children who had bronchopulmonary dysplasia with current respiratory symptoms than in the children who had not had idiopathic respiratory distress syndrome ($p<0{\cdot}05$). The differences between the children who had had bronchopulmonary dysplasia with and without current symptoms was not significant. The mean $\overline{dR_{rs}/df}$ was significantly lower in the children who had bronchopulmonary dysplasia with current symptoms than in those without ($p<0{\cdot}01$). Both groups had a significantly lower mean $\overline{dR_{rs}/df}$ than the children who did not have idiopathic respiratory distress syndrome ($p<0{\cdot}01$).

Of the children who had bronchopulmonary dysplasia, moderate bronchial hyper-responsiveness was found in one of six with current symptoms and

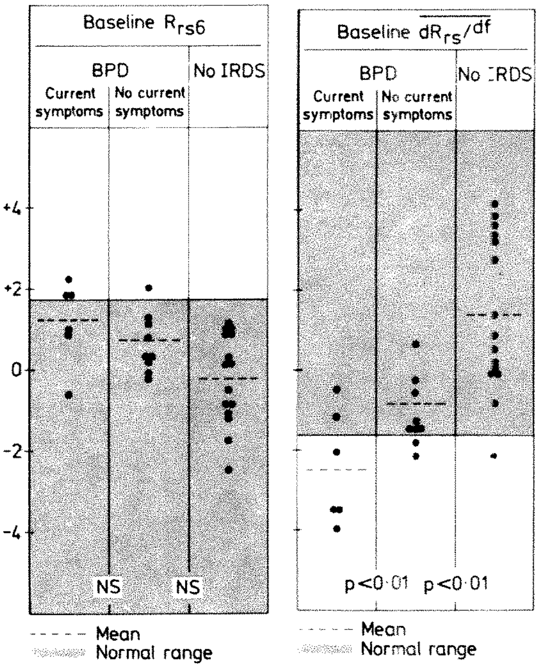

Figure *Children who were born prematurely and had bronchopulmonary dysplasia compared with healthy children born prematurely who did not have idiopathic respiratory distress syndrome.*

in one child who had had symptoms in the past (table 1). Bronchial hyper-responsiveness was present in two children who never had noticeable respiratory symptoms after discharge from hospital. Moderate bronchial hyper-responsiveness was found in three children who had not had idiopathic respiratory distress syndrome (table 2).

There was no significant difference in mean $PD_{40}R_{rs6}$ to histamine between the children who had bronchopulmonary dysplasia and the subjects who had not had idiopathic respiratory distress syndrome, or between these two groups and the reference population.

Discussion

Severe lung injury during early life may affect the growth and development of the respiratory system.[3 4] Injury to immature lung structures may cause subsequent damage of peripheral airways.[4] Important long term abnormalities that have been described in children who had been ventilated for idiopathic respiratory distress syndrome are airway obstruction—that is increased airway resistance, diminished dynamic lung volume and expiratory flow, increased residual volume, as well as bronchial hyper-responsiveness to histamine, methacholine, or exercise.[5-8] These studies also reported a higher incidence of dysfunction when idiopathic respiratory distress syndrome had been complicated by bronchopulmonary dysplasia. In children with asthma, both airway obstruction and bronchial hyper-responsiveness are independent indicators of chronic lung disease.[25] Our purpose was to investigate the incidence of these indicators in children who had had idiopathic respiratory distress syndrome with bronchopulmonary dysplasia. We therefore compared children of school age who were born prematurely and had idiopathic respiratory distress syndrome with severe bronchopulmonary dysplasia, with children born prematurely who had not had idiopathic respiratory distress syndrome.

We have used forced oscillation to distinguish between central and peripheral airway obstruction. Forced oscillation measurements can be obtained in children from about 2½ years of age because only passive cooperation is needed. The within subject reproducibility of consecutive forced oscillation measurements is similar to that of forced expiratory flow volume curves.[23] Forced oscillation appears to be as sensitive in measuring bronchial hyper-responsiveness as FEV_1.[23] The results of forced oscillation testing showed that central and peripheral airway function were abnormal in most children with current symptoms. Of the children who were free of symptoms, however, one had abnormal measurements.

Many children who had no respiratory symptoms after having had bronchopulmonary dysplasia had values of $\overline{dR_{rs}/df}$ that were at the lower limit of the normal range. This resulted in a significantly lower mean $\overline{dR_{rs}/df}$ than in the healthy controls born at term from the reference population[19] and in children who were born prematurely but did not have idiopathic respiratory distress syndrome. This suggests that idiopathic respiratory distress syndrome and bronchopulmonary dysplasia may have caused subtle damage to peripheral airways that has not been shown by previous studies.

Premature birth not followed by idiopathic respiratory distress syndrome did not result in abnormal lung function according to the indicators used in this study. Although the premature babies who did not develop idiopathic respiratory distress syndrome were on average four weeks more mature at birth than those with idiopathic respiratory distress syndrome, it seems unlikely that this difference has influenced our results.

Our findings about central airway function are in agreement with those in most other studies.[9-15] Peripheral airway function has not to our knowledge been assessed.

There was no difference in the incidence of bronchial hyper-responsiveness between children who had bronchopulmonary dysplasia, irrespective of current symptoms, and children born prematurely who had not had idiopathic respiratory distress syndrome. Our results are therefore different from those of Smyth *et al*,[5] Bertrand *et al*,[6] and Wheeler *et al*,[7] all of whom found a high incidence of slight to moderate bronchial hyper-responsiveness after idiopathic respiratory distress syndrome or bronchopulmonary dysplasia. Our findings indicate that prematurity or severe lung injury in early life do not lead to the degree of bronchial hyper-responsiveness that is commonly seen in children with asthma.[24] Todisco *et al* found that the responsiveness to methacholine in premature infants and in survivors of idiopathic respiratory distress syndrome was within the normal range by school age.[26] It is not clear why some children in this study had bronchial hyper-responsiveness. There was no association with a family history of asthma in first or second degree relatives, nor with baseline airway calibre, though bronchial hyper-responsiveness has been associated with atopy.[27] We were unable to study atopy, but because three of the four children with bronchial hyper-responsiveness had no current symptoms atopy seems unlikely.

Viral infections may also cause transient bronchial hyper-responsiveness.[28] Although we tried to exclude patients who had had viral respiratory infections in the two weeks preceding the measurements, we cannot be sure that some of the hyper-responsive children may have had slight infections that were not noticed and induced transient bronchial hyper-responsiveness.

We conclude that about 40% of the children who had survived idiopathic respiratory distress syndrome with severe bronchopulmonary dysplasia had current respiratory symptoms into school age. Most subjects who continued to have symptoms after idiopathic respiratory distress syndrome and bronchopulmonary dysplasia had increased R_{rs6} or decreased $\overline{dR_{rs}/df}$, indicating obstruction in different parts of the airway. Although central and peripheral airway calibre in most asymptomatic children who had bronchopulmonary dysplasia was within the normal range, low $\overline{dR_{rs}/df}$ values suggest subtle peripheral airway damage. Whether this will result in airway disease in future is uncertain. Premature birth without idiopathic respiratory distress syndrome did not result in abnormal values of R_{rs6} or $\overline{dR_{rs}/df}$. It is therefore unlikely that immaturity of the respiratory system without injury will lead to abnormal development of the airways. Our data do not allow us to differentiate between bronchopulmonary dysplasia and uncomplicated idiopathic respiratory distress syndrome as causes of airway damage because only children who suffered from bronchopulmonary dysplasia were included in the study. In the light of the findings in prematurely born children who did not develop idiopathic respiratory distress syndrome, however, we speculate that the severity of lung injury is an important determinant of the risk of long term airway damage. We also conclude that bronchial hyper-responsiveness was not a feature of children with idiopathic respiratory distress syndrome and bronchopulmonary dysplasia.

Whether treatment of respiratory symptoms in children who had severe neonatal lung damage will have an effect on airway function later in life is unknown. In view of our findings and those of others, it can be speculated that such treatment should be recommended.

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References

[1] Cudmore RE, Emery JL, Mithal A. Postnatal growth of bronchi and bronchioles. *Arch Dis Child* 1962;**37**:481–2.

[2] Hogg JC, Williams J, Richardson JB, Macklem PT, Thurlbeck WM. Age as a factor in the distribution of lower airway conductance and in the pathologic anatomy of obstructive lung disease. *N Engl J Med* 1970;**282**:1283–7.

[3] Thurlbeck WM. Growth, development and ageing of the lung. In: Scadding JG, Cumming G, Thurlbeck WM, eds. *Scientific foundations of respiratory medicine.* 1st ed. London: Heinemann, 1982:91–110.

[4] Chaney HR, Mallory GB, Motoyama EK, Mutich RL. Functional development of the lung in infants with severe bronchopulmonary dysplasia. *Am Rev Respir Dis* 1987;**135**:A126.

[5] Smyth JA, Tabachnik ET, Duncan WJ, Reilly BJ, Levison H. Pulmonary function and bronchial hyperreactivity in long-term survivors of bronchopulmonary dysplasia. *Pediatrics* 1981;**68**: 336–40.

[6] Bertrand JM, Riley S, Popkin J, Coates AL. The long-term pulmonary sequelae of prematurity: the role of familial airway hyperreactivity and the respiratory distress syndrome. *N Engl Med* 1985;**312**:742–5.

[7] Wheeler WB, Castile RG, Brown ER, Wohl MEB. Pulmonary function in survivors of prematurity. *Am Rev Respir Dis* 1984;**129**:A218.

[8] Bader D, Ramos AD, Lew CD, Platzker ACG, Stabile MW, Keens TG. Childhood sequelae of infant lung disease: exercise and pulmonary function abnormalities after bronchopulmonary dysplasia. *J Pediatr* 1987;**110**:693–9.

[9] Lamarre A, Linsao L, Reilly BJ, Swyer PR, Levison H. Residual pulmonary abnormalities in survivors of idiopathic respiratory distress syndrome. *Am Rev Respir Dis* 1973;**108**: 56–61.

[10] Coates AL, Bergsteinsson H, Desmond K, Outerbridge EW, Beaudry PH. Long-term pulmonary sequelae of premature birth with and without idiopathic respiratory distress syndrome. *J Pediatr* 1977;**90**:611–6.

[11] Heldt GP, McIlroy MB, Hansen TN, Tooley WH. Exercise

performance of the survivors of hyaline membrane disease. *J Pediatr* 1980;**96**:995–9.

[12] Borkenstein J, Borkenstein M, Rosegger H. Pulmonary function studies in longterm survivors with artificial ventilation in the neonatal period. *Acta Paediatr Scand* 1980;**69**:159–63.

[13] Stahlman M, Hedvall G, Lindstrom D, Snell J. Role of hyaline membrane disease in production of later childhood lung abnormalities. *Pediatrics* 1982;**69**:572–6.

[14] Mansell AL, Driscoll JM, James LS. Pulmonary follow-up of moderately low birth weight infants with and without respiratory distress syndrome. *Am Rev Respir Dis* 1985;**131**:A249.

[15] Biscoll DJ, Kleinberg F, Heise CT, Staats BA. Cardiorespiratory function in asymptomatic survivors of neonatal respiratory distress syndrome. *Mayo Clin Proc* 1987;**62**:695–700.

[16] Northway WH, Rosan RC, Porter DY. Pulmonary disease following respiratory therapy of the hyaline-membrane disease; bronchopulmonary dysplasia. *N Engl J Med* 1967;**276**:357–68.

[17] Toce SS, Farrell PM, Lewitt LA, Samuels DP, Edwards DK. Clinical and roentgenological scoring systems for assessing bronchopulmonary dysplasia. *Am J Dis Child* 1984;**138**:581–5.

[18] Kerrebijn KF, Hoogeveen-Schroot HCA, van der Wal MC. Chronic nonspecific respiratory disease in children, a five year follow-up study. *Acta Paediatr Scand* 1977;**261**:1–72.

[19] Duiverman EJ, Clement J, van de Woestijne KP, Neijens HJ, van den Bergh ACM, Kerrebijn KF. Forced oscillation technique; reference values for resistance and reactance over a frequency spectrum of 2–26 Hz in healthy children aged 2·3–12·5 years. *Bull Europ Physiopathol Respir* 1985;**21**:171–8.

[20] Clément J, Lándsèr FJ, van de Woestijne KP. Total resistance and reactance in patients with respiratory complaints with and without airways obstruction. *Chest* 1983;**83**:215–20.

[21] Mead J. Contribution of compliance of airways to frequency dependent behaviour of lungs. *J Appl Physiol* 1969;**26**:670–3.

[22] Harf A, Decremer M, Zin W, Milic-Emili J, Chang HK. Respiratory resistance in dogs by the single-breath and forced oscillation methods. *J Appl Physiol* 1985;**59**:262–5.

[23] Duiverman EJ, Neijens HJ, van der Snee-van Smaalen M, Kerrebijn KF. Comparison of forced oscillometry and forced expirations for measuring dose-related responses to inhaled methacholine in asthmatic children. *Bull Europ Physiopathol Respir* 1986;**22**:433–6.

[24] Duiverman EJ, Neijens HJ, van Strik R, Affourtit MJ, Kerrebijn KF. Lung function and bronchial responsiveness in children who had infantile bronchiolitis. *Pediatr Pulmonol* 1987;**3**:38–44.

[25] Martin AJ, Landau LI, Phelan PD. Lung function in young adults who had asthma in childhood. *Am Rev Respir Dis* 1980;**122**:609–15.

[26] Todisco T, Dottorini M. Outcome for survivors from respiratory distress syndrome. *Lancet* 1987;**i**:1213.

[27] Neijens HJ, Duiverman EJ, Kerrebijn KF. Bronchial responsiveness in children. *Pediatr Clin North Am* 1983;**30**:829–46.

[28] Empey DW, Laitinen LA, Jacobs L, Gold WM, Nadel JA. Mechanics of bronchial hyperreactivity in normal subjects after upper respiratory tract infection. *Am Rev Respir Dis* 1976;**113**:131–9.

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Effect of maternal hyperalimentation on intrauterine growth retardation

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SUMMARY The effect of maternal hyperalimentation on intrauterine growth retardation was studied by performing unilateral uterine vessel ligation on day 17 in Wistar rats. Test animals were given food supplements by gavage for three days postoperatively whereas control animals received saline. The caloric intake and weight gain of the test dams was significantly greater than that of the controls from days 17 to 20 but not over the longer period, day 14 to 23. The survival and growth of fetuses in the unoperated horn were similar in test and control rats. Fetal survival in the ligated horn was doubled by maternal food supplementation but the growth and development of the survivors were not improved.

Intrauterine growth retardation (IUGR) has many causes and in human pregnancy maternal vasculopathy is responsible for roughly one third of all cases.[1] Starvation during pregnancy also leads to IUGR and in undernourished populations caloric supplementation during pregnancy can cause a modest increase of birth weight.[2] There is a limited therapeutic repertoire available to the clinician who has to deal with IUGR[3] and we were therefore interested to see if maternal food supplementation could benefit fetal outcome in pregnancies with IUGR due to vascular disease. The Wigglesworth model of intrauterine growth retardation in the rat is an appropriate example of uterine vasculopathy and this study examined the effect of maternal food supplementation on fetal outcome in this model.[4]

Materials and methods

ANIMALS

Primiparous Wistar rats, 80 to 120 days old from the University of Nottingham animal house, were used. First, in order to test if the completely absorbable diet would sustain pregnancy as well as a conventional diet pregnancy outcome was compared between four animals that were fed a conventional laboratory diet (RMH-GS, Hope Farms, The Netherlands) during their whole gestation and four animals that were switched to a completely absorbable powder diet (Precitene-N, Sanofi, Belgium) from day 14 of pregnancy. Percentage weight increase per 24 hours and caloric uptake in kcal/kg/24 hours were recorded from day 14 by direct weighing. At day 22 the dams were sectioned under pentobarbitone anaesthesia. Litter size and fetal survival rate were compared between control and test groups. Mean values for daily caloric uptake, daily weight gain, fetal body weight, and placental weight were calculated per dam.

Then 17 primigravid English Wistar rats with known duration of pregnancy were given Precitene-N from day 14. At day 17 the uterine vessels were ligated unilaterally.[4] Ten dams received food supplements from day 17 to 19 inclusive. Supplements were given as concentrated solutions of 20 g Precitene-N in 12 ml water. According to the technique described by Waynforth, the solution was administered by the intragastric route using a 15 gauge needle with a bulbous tip on a 10 ml syringe.[5] The rats were held firmly by the skin of the neck so that the head was immobile and the mouth opened. The needle, attached to the syringe, was then passed into the stomach. One to two ml of the Precitene-N solution were given at a time and maximum daily amounts were 16 ml. Equal amounts of physiologic saline were given in the same way to seven control rats. Meanwhile test and control dams always had free access to Precitene-N powder and water. Maternal body weight and total food uptake were recorded daily. The Wilcoxon matched pairs signed ranks test was used to evaluate the difference between food supplemented and sham treated dams.

At day 23 the dams were anaesthetised with intraperitoneal nembutal and delivered operatively. Fetal blood was sampled from the axillary vessels while the placental circulation was still intact.[6] Blood was pooled from all fetuses within a single uterine horn and stored at −20°C until analysed. Fetuses and placentas were then weighed to accuracies of 0·1 g and 1 mg, respectively. From each fetus liver and pancreas were removed in toto, weighed, and kept for further analysis. Fetal livers were stored at −20°C until analysed.

LABORATORY METHODS

Plasma somatomedin like activity was measured by bioassay in which ^{35}S sulphate uptake by fetal rat costal cartilage was the test system and the reference standard was a pool of adult rat plasma (5% v/v).[7]

Each fetal liver was homogenised in deionised water in a Potter-Elvehjeim homogeniser. Hepatic protein concentration was determined using bovine serum albumin as a standard.[8] Deoxyribonucleic acid (DNA) was measured using calf thymus DNA (Sigma Chemical, Dorset) as a standard.[9] Aliquots of liver homogenate were extracted twice with 0·5 N perchloric acid for 30 minutes at 80°C with a yield of 95%. As suggested, acetaldehyde solution (2·05%) was added to the diphenylamine reagent immediately before use.[10]

Fetal pancreas was fixed in Bouin's solution and embedded in paraffin wax. Tissue sections were stained immunocytochemically for insulin and pancreatic polypeptide by the unlabelled antibody enzyme method.[11] Two sections from each tissue block were used for the morphometric determination of pancreatic B cell density.[12] The number of points counted per pancreas was 51 200 resulting in standard errors of 4% per fetus and 0·6 to 2·0% for a group of fetuses within a litter.[13] From the pancreatic B cell volume density, pancreatic weight, and body weight, the relative weight of B cells in the fetus was calculated.

STATISTICAL ANALYSES

Differences in maternal responses to the two diets were compared by Student's *t* test. Parameters of fetal outcome such as survival or plasma somatomedin like activity were averaged per uterine horn. The Mann-Whitney test was used to compare these parameters between ligated and non-ligated horns in food supplemented and sham treated dams. Fetal body weight and organ characteristics should have been subjected to analysis of variance with hierarchial classification[14] but this was not possible because of the high fetal death rate in the ligated horn of the sham supplemented dams.

Results

The chemical composition of the conventional laboratory diet and the completely absorbable powder diet differed[15] but were similar and each fulfilled the estimated requirements for adequate rat nutrition.[16] Pregnant rats fed the absorbable powder diet from day 14 to 22 took on average (SEM) 197 (9) kcal/kg/24 hours (0·82 (0·04) MJ) which was significantly less than that of those fed conventionally: 273 (9) (1·14 (0·04) MJ) ($p<0·005$). Despite this the mean (SEM) weight gain of the two quartets was similar: 2·7 (0·1)% increase/day, as were the litter size (13 (2) *v* 13 (1)), fetal survival rate (100% *v* 98%), fetal body weight (4·4 (0·1) g each), and placental weight (453 (25) *v* 443 (25) mg). It was concluded that the pregnant rats thrived equally on either diet.

In the definitive experiment where dietary intake was supplemented by gavage after unilateral uterine vessel ligation on day 17 of pregnancy, the food supplemented dams both received more calories and gained more weight from days 17 to 20, but when similar analyses were performed over the period 14 to 23 days no significant difference between the groups could be detected (table 1). Dietary intake in the first three postoperative days was significantly down in both groups, more so in the sham treated.

Comparisons of the effects of uterine vessel ligation and maternal food supplementation are presented in table 2. Considering first the control horns: there was no significant difference between the food supplemented and sham supplemented groups in any parameter studied. The effect of

Table 1 *Mean (SE) maternal nutrition and weight gain after uterine vessel ligation on day 17 of food supplemented or sham treated pregnant rats*

Treatment	*Caloric uptake (kcal/kg/24 hours)*		*Daily weight gain (% increase/24 hours)*	
	Day 14 to 23	*Day 17 to 20*	*Day 14 to 23*	*Day 17 to 20*
Food supplemented rats (n=10)	197 (7)	160 (10)*	+1·3 (0·2)	+1·2 (0·3)*
Sham treated rats (n=7)'	184 (9)	127 (10)	+0·9 (0·1)	−0·2 (0·2)

Significance of difference of food supplemented *v* sham treated: *$p<0·05$.

Table 2 *Mean (SE) fetal outcome on day 23 after unilateral uterine vesel ligation on day 17 of pregnant rats subsequently given food supplements or sham supplements*

	Sham supplement		*Food supplement*	
	Ligated horn	*Control horn*	*Ligated horn*	*Control horn*
No survivors/dam	0·6 (0·3) [7]*	4·9 (0·5) [7]	2·0 (0·5) [10]	4·4 (0·5) [10]
Survival rate (%)	13 (7) [7]	97 (3) [7]	40 (9) [10]	83 (4) [10]
Body weight (g)	4·5 (0·5) [4]	5·3 (0·1) [34]	4·5 (0·2) [20]**	5·6 (0·1) [10]
Placental weight (mg)	459 (47) [4]	498 (14) [34]	452 (26) [20]**	529 (16) [44]
Pancreas weight (mg)	22 (2) [4]	27 (1) [34]	23 (1) [20]**	28 (1) [44]
Liver weight (mg)	255 (40) [4]	345 (12) [34]	233 (15) [20]**	346 (9) [44]
Liver DNA (μg)	154 (17) [4]	173 (10) [34]	136 (10) [20]**	189 (9) [44]
Liver protein/DNA	317 (22) [4]	364 (10) [34]	322 (20) [20]	327 (14) [44]
Plasma somatomedin like activity (U/ml)	0·98 (0·17) [3]	1·35 (0·24) [5]	1·06 (0·13) [8]**	2·18 (0·44) [8]

The number of observations is shown in square brackets.
Significance of difference for fetuses in the ligated horns of fed *v* sham supplemented mothers: *p<0·05, and for fetuses in the ligated *v* control horns of the same supplement group: **p<0·05.

uterine vessels ligation in the food supplemented group was to halve the number of survivors per horn and cause a small but significant reduction in their body weight, a significant reduction in plasma somatomedin like activity, placenta, pancreas, and liver weights but no significant change in the hepatic protein:DNA ratio. In the food supplemented group the mean (SEM) pancreatic B cell volume density of the survivors in the ligated horn was similar to that in the control horn (1·12 (0·09) and 1·41 (0·14)%, respectively) as was the fractional contribution of B cell weight to body weight (59 (4) and 69 (6) μg/g, respectively).

In stark contrast, ligation in the sham supplemented group resulted in the death of all or all but one of the fetuses in the uterine horn. Four fetuses survived in seven ligated uterine horns. The body, organ, and biochemical characteristics of these four fetuses had the same relation to the fetuses in the control horn as had been noted between the fetuses in the ligated and control horns of the food supplemented group.

It was concluded that food supplementation protected against the fetal mortality associated with uterine vessel ligation but had no effect on the morbidity.

Discussion

These experiments were designed to study the effect of maternal food supplementation on fetal outcome in the presence of restricted uteroplacental circulation. The rat was chosen as the experimental model because of the large experience with uterine vessel ligation in this species but it was hoped that the results might have more general biological implications.

Nutritional supplementation can be given enterally or parenterally. Pilot experiments showed that the combination of laparotomy for uterine vessel ligation with constant intravenous infusion led to an unacceptably high maternal mortality, hence gavage was chosen to supplement maternal intake. The completely absorbable diet Precitene-N was used because it can be dissolved for gavage feeding as well as being eaten by rats in powder form. Although Precitene-N differs in detail from commercial rat chow, there are many similarities and it contains all the recommended nutrients for the rat. The preliminary experiment established that both mother and fetus fared equally well on either diet. Gavage was a satisfactory technique for food supplementation because the rat has a poorly developed vomiting reflex.[17] Under nembutal anaesthesia up to 6 ml Precitene-N could be given and supplementation of conscious rats with 1–2 ml at a time was easy. In general the rat eats according to its energy requirement and forced feeding is accompanied by reduced spontaneous food intake. In the present experiments food supplementation was important from day 17 to 20 when spontaneous food intake was low in the immediate postoperative period. At this time the food supplemented rats ingested roughly 30% more than the sham treated controls and they continued to gain weight, whereas over the whole study period of 14 to 23 days there was no significant difference in energy intake or weight gain of food supplemented or sham supplemented animals.

The experiments were designed to permit analysis of the effects of maternal food supplementation and uterine vessel ligation separately and together. Gavage by itself had no effect, adverse or beneficial, on fetal outcome. Uterine vessel ligation produced a high fetal mortality with only one fetus maximum surviving in the experimental horn and three out of seven dams losing all the fetuses in the ligated horn. This result was clearly related to the colony of animals because the same investigator (F De P) had earlier performed identical operations on an English colony of Wistar rats with an average 54% fetal survival and 92% of the mothers having one or more living fetuses. The growth and composition of the four surviving fetuses was similar to that of the fetuses in the ligated horn of the food supplemented group; the difference between the two being in the number of survivors, on average two fetuses out of four survived in the food supplemented group and no food supplemented dam failed to have at least one survivor in the ligated horn. Thus food supplementation improved survival of fetuses experiencing uterine vascular impairment but had no effect on the size of the survivor. It remains a matter of speculation as to what effect food supplementation might have had on a colony in which spontaneous survival in a ligated horn was higher.

Analyses of fetal body and organ weight and composition gave similar results between the control and test animals for ligated and non-ligated horns, respectively. Fetal growth was most advanced in the non-ligated horns of the food supplemented rats where the fetuses were the most favoured by the experimental design. They also had the highest plasma somatomedin like activity, total liver DNA, and relative weight of B cells. These findings are compatible with the idea that the relative weight of B cells reflects the growth capacity of the fetus and they confirm the positive correlation between body weight and plasma somatomedin like activity described earlier.[18] Liver weight was strikingly lower in the test fetuses of both food supplemented and non-supplemented mothers confirming the findings of others.[19] The extent of the liver weight reduction, roughly 25%, was too great to be explained by glycogen alone. Analyses of protein and DNA showed preservation of the protein:DNA ratio in all subgroups of fetuses and the reduction of hepatic weight was therefore due to fewer, not smaller, cells. The similarity of placental weight in the subgroups indicates that maternal hyperalimentation does not influence fetal outcome by stimulating placental growth.

Extrapolation of these results to any other species would be inappropriate apart from the general and clear cut observation that fetal outcome can be importantly affected by the plane of maternal nutrition, particularly in a postoperative period late in gestation.

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References

[1] The causes of small-for-dates birth. General discussion. In: Elliot K, Knight J, eds. *Size at birth*. Amsterdam: Associated Scientific Publishers, 1974:383–97.

[2] Habicht JP, Yarbrough C, Lechtig A, Klein RE. Relation of maternal supplementary feeding during pregnancy to birth weight and other sociobiological factors. In: Winick M, ed. *Nutrition and fetal development*. New York: Wiley, 1974: 127–45.

[3] Nicolaides KH, Campbell S, Bradley RJ, Bilardo CM, Soothill PW, Gibb D. Maternal oxygen therapy for intrauterine growth retardation. *Lancet* 1987;**i:**942–5.

[4] Wigglesworth JS. Experimental growth retardation in the fetal rat. *Journal of Pathology and Bacteriology* 1964;**88:**1–13.

[5] Waynforth HB. Injection techniques. In: Waynforth HB, ed. *Experimental and surgical techniques in the rat*. London: Academic Press, 1980.

[6] Kervran A, Gilbert M, Girard JR, Jost A. Effect of environmental temperature on glucose-induced insulin response in the newborn rat. *Diabetes* 1976;**25:**1026–30.

[7] Hill DJ, Andrews SJ, Milner RDG. Cartilage response to plasma and somatomedin activity in rats related to growth before and after birth. *J Endocrinol* 1983;**90:**133–42.

[8] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. *J Biol Chem* 1951:**193:** 165–75.

[9] Burton K. A study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem J* 1956;**62:**315–23.

[10] Giles KW, Myers A. An improved diphenylamine method for the estimation of deoxyribonucleic acid. *Nature* 1965:**206:**93.

[11] Sternberger LA, Handy PH, Curculis JJ, Meyer HC. An unlabeled antibody method of immunochemistry. *J Histochem Cytochem* 1976;**18:**315–40.

[12] De Prins FA, Van Assche FA. Intrauterine growth retardation and development of endocrine pancreas in the experimental rat. *Biol Neonate* 1982:**41:**16–21.

[13] Weibel ER. Point counting methods. In: Weibel ER, ed. *Stereological methods*. Vol I. London: Academic Press, 1979:101–61.

[14] Li CC. Hierarchical classifications. In: Li CC, ed. *Introduction to experimental statistics*. New York: McGraw-Hill Book Company, 1964:75–83.

[15] De Prins F. Fetal consequences of growth retardation on a vascular basis. A study in an animal model. Leuven: University of Leuven, 1987. (PhD thesis)

[16] Committee on animal nutrition. National requirements of the laboratory rat. *National Academy of Sciences, Nutrient requirements of laboratory animals*. Washington DC: Office of Publications, 1978:7–37.

[17] Briggs GB, Dehme FW. Toxicology. In: Baker HJ, Lindsey JR, Weisbroth SH, eds. *The laboratory rat*. Vol II. *Research applications*. New York: Academic Press, 1980:103–18.

[18] De Prins FA, Hill DJ, Fekete M, Robson D, Van Assche FA, Milner RDG. Reduced plasma somatomedin activity and sulphate incorporation by costal cartilage in vitro during experimental growth retardation in the fetal rat. *Pediatr Res* 1984:**18:**1100–4.

[19] Roux JM, Tordet-Caridroit C, Chanez C. Studies on experimental hypotrophy in the rat. I. Chemical composition of the total body and some organs in the rat foetus. *Biol Neonate* 1976;**29:**262–6.

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Neonatal ovarian cysts: therapeutic dilemma

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SUMMARY Seven cases of neonatal ovarian cysts that presented over the past seven years were studied. Complications included torsion and rupture and usually occurred in cysts more than 5 cm in diameter. Surgical removal, either oophorectomy or cystectomy, was the treatment of choice. Because even cystectomy results in loss of normal ovarian tissue, and because spontaneous regression of cysts less than 5 cm in diameter can occur, a more conservative approach is now proposed. Regular ultrasonography alone is recommended if the cysts are less than 5 cm in diameter, and aspiration of the cysts followed by regular ultrasonographs if the cysts are more than 5 cm in diameter. Operation should be reserved for recurrent cysts or for those with complications. Cysts diagnosed antenatally may be aspirated in utero if there are signs of thoracic compression.

Neonatal ovarian cysts are being diagnosed more often now that routine ultrasonography is carried out antenatally and postnatally. A truly cystic abdominal mass in a baby girl is most likely to be an ovarian cyst, although duplication cyst or mesenteric cyst should be considered in the differential diagnosis.

There is controversy about the best treatment for these cysts, opinions ranging from oophorectomy to follow up by ultrasonography alone. We propose a regimen the main objective of which is to avoid unnecessary operation especially in those patients with bilateral cysts.

Case reports

CASE 1

The infant was born prematurely by emergency caesarean section at 33 weeks' gestation weighing 2700 g. The mother had insulin dependent diabetes and had been treated for thyrotoxicosis by thyroidectomy six years previously. She had also had one spontaneous abortion and two stillbirths.

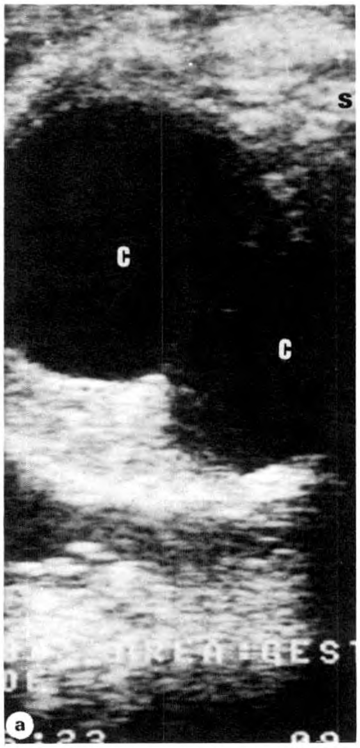
A routine antenatal ultrasound scan at 32 weeks showed a cystic mass in the baby's abdomen. This was confirmed when she was 2 weeks old as a cystic abdominal mass measuring 4×3 cm. At laparotomy on the following day a cyst arising from the right ovary was enucleated. The uterus and left ovary were normal. On histological examination the cyst was found to be a simple follicular cyst with evidence of luteinisation. The patient made an uneventful recovery.

CASE 2

The infant was born by normal spontaneous vaginal delivery at 35 weeks' gestation weighing 3200 g. The mother had had two spontaneous abortions and two pregnancies terminated; she was also a heroin addict. Nine days before delivery two cystic central abdominal masses that were separate from the kidneys were noted on ultrasound scan (fig 1a). A scan performed when she was one day old (fig 1b) confirmed the presence of cystic structures measuring 8×4 cm and 5×4 cm diameter, respectively. At operation bilateral ovarian cysts were found and bilateral cystectomies performed. Both cysts were unilocular and contained clear yellow fluid. On histological examination the cyst walls were found to be thin and contained some ovarian tissue with a few follicular cysts. The patient made a satisfactory recovery and was discharged home three weeks after the operation.

CASE 3

The infant was born by normal vaginal delivery at full term weighing 3300 g. The mother was well. An ultrasound examination was performed at 38 weeks' gestation for a possible abruptio placentae and showed a cystic lesion 6 cm in diameter in the right side of the baby's abdomen. This was confirmed by a scan performed when she was 1 day old, and the lesion contained a few fine septa. At laparotomy on day 8 a mobile, right sided ovarian cyst was found, which had twisted twice at the junction of the right fallopian tube and the uterus. A right salpingo-oophorectomy was performed. The left ovary was



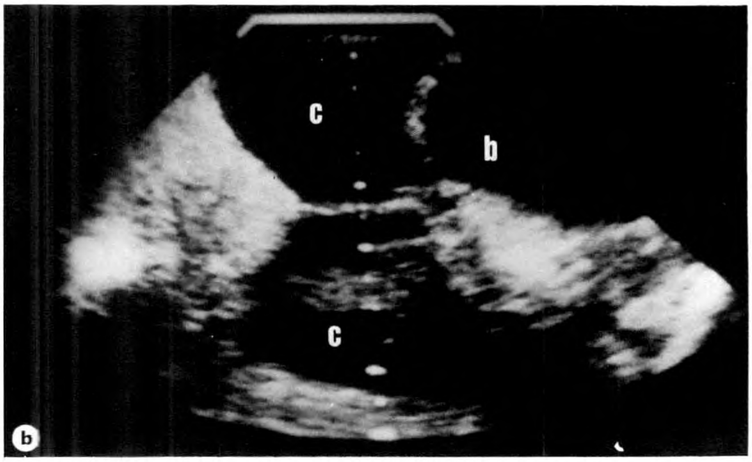


Fig 1a *Transverse scan of fetal abdomen (case 2) showing two cystic masses (c); (s)=spine.*
Fig 1b *Postnatal oblique lower abdominal scan (case 2) confirming the presence of cysts (c) separate from bladder (b).*

normal and contained a few small follicular cysts. An infarcted cyst was confirmed on histological examination. The cyst was thickened (1 cm) in places and contained dark brown fluid. The patient was discharged on the sixth day postoperatively after an uneventful recovery.

CASE 4

The infant was born by spontaneous vaginal delivery at 27 weeks' gestation weighing 2000 g. She had many problems in the immediate neonatal period including respiratory distress syndrome that required ventilation, a grade II intraventricular haemorrhage on the left, jaundice, and haemolytic anaemia that required two transfusions. No cause was found for the anaemia but during investigation an ultrasound examination was performed to exclude polysplenia. The spleen was normal but a cystic lesion in the abdomen was found. A laparotomy was performed at 18 weeks at which a twisted cyst of the right ovary 5 cm in diameter was found together with a cyst of the left ovary 2·5 cm in diameter. Right salpingo-oophorectomy and drainage of the left cyst with biopsy were carried out.

On histological examination the specimen from the left cyst showed normal ovarian tissue whereas the right cyst was totally infarcted with areas of calcification within it and only a simple flattened epithelial lining; it contained brown necrotic material. The patient was discharged home 13 days postoperatively.

CASE 5

The infant was born by normal vaginal delivery at full term weighing 3800 g after an uneventful pregnancy. The mother was well. At the age of 5 months the baby was admitted to hospital with abdominal distension and a three month history of intermittent abdominal swelling that had caused dyspnoea at night. Ultrasound examination showed a unilocular cyst 10 cm in diameter in the centre of the abdomen, separate from the kidneys and the bladder. At laparotomy a large left sided ovarian cyst was found that had twisted two and a half times round the left fallopian tube. A cystectomy was performed, and an ovarian remnant and the left fallopian tube were left behind. The right adnexa was normal. On histological examination the cyst contained dysplastic ovarian parenchyma with a few oogonia and an occasional primary graafian follicle. The patient was discharged home six days postoperatively.

CASE 6

The infant was born by normal vaginal delivery at full term weighing 3300 g. The mother was well. A routine antenatal ultrasound scan at 32 weeks' gestation showed a cystic mass 4×3 cm in diameter in the left side of the abdomen separate from the kidneys. This was confirmed when she was 5 weeks old, but this time the lesion lay on the right side of the abdomen. The mass was not palpable. By 25 weeks of age the mass had become palpable and repeat ultrasound examination confirmed an increase in its size to 6×5 cm. A large cyst of the left ovary was removed at laparotomy. The right ovary was normal. She also had a malrotation of the bowel and this was corrected. On histological examination the ovarian mass was found to be a simple follicular cyst. The patient made an uneventful recovery.

CASE 7

The infant was born by normal vaginal delivery at full term weighing 3100 g. The mother was well, but the pregnancy was complicated by hydramnios. A routine antenatal ultrasound scan at 34 weeks' gestation showed a cystic mass 7×5 cm in diameter in the abdomen (fig 2). This was confirmed when she was 1 day old, the mass having decreased in size to 6×4 cm. The patient was otherwise normal, in particular there was no evidence of gut atresia to account for the hydramnios. A scan two weeks later suggested that the mass was multilocular. At laparotomy bilateral ovarian cysts were found, the left being about twice the size of the right. The right cyst was chronically twisted and a right salpingo-oophorectomy was performed, together with a left cystectomy. On histological examination the right cyst was found to be multilocular and follicular, and the left unilocular and follicular. There were no postoperative complications and the patient was discharged home three days later.

Discussion

Before the introduction of ultrasonography, ovarian cysts in neonates were thought to be rare and could only be diagnosed postnatally.[1-3] Only 71 cases were reported before 1976.[4] Cysts were only discovered if they were palpable or became symptomatic, but, with ultrasonography asymptomatic ovarian cysts could be diagnosed.[5 6] Ovarian cysts can now be diagnosed antenatally, and 11 such cases had been reported up to 1987.[7 8] The pathogenesis of neonatal ovarian cysts is unknown. Examination of the ovaries of 121 children at necropsy showed that there was more pronounced luteinisation of the theca interna in the neonatal ovaries studied when compared with the older age groups[9]; this was subsequently confirmed by a study of normal neonatal and infantile ovaries.[10] Another series of 332 necropsies of stillbirths and neonatal deaths showed that 34% had small follicular cysts within 28 days of birth.[11] It is almost certain, therefore, that the stimulus for the formation of neonatal ovarian cysts is chorionic gonadotrophin that stimulates the fetal ovary during pregnancy. Luteinising cysts are occurring increasingly more often in babies whose mothers were diabetic (case 1) or had toxaemia, or maternal isoimmunisation, because these conditions are all associated with raised concentrations of human chorionic gonadotrophin.[12 13] There is also an increasing incidence in premature infants, probably because of their greater sensitivity to human chorionic gonadotrophin (cases 1, 2, and 4).[14] On

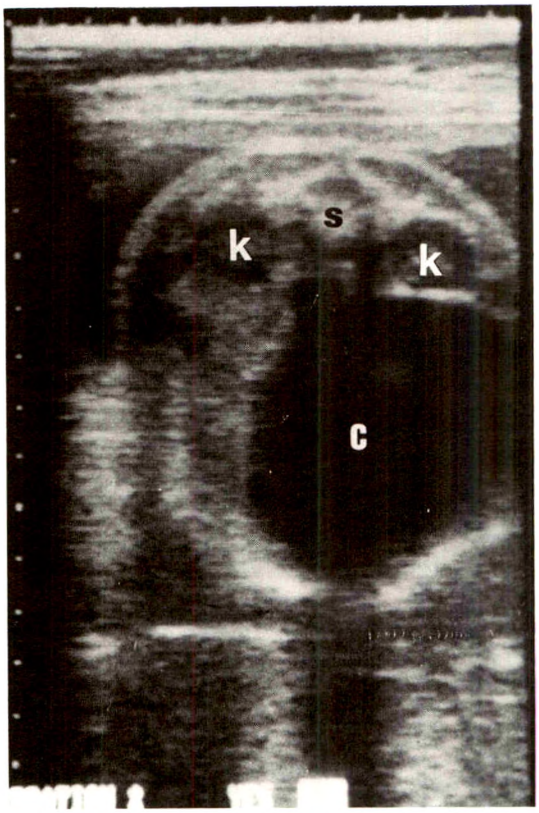


Fig 2 *Transverse section of fetal abdomen (case 7). Large abdominal cyst (c) is shown separate from kidneys (k) and spine (s).*

histological examination the cysts are follicular or luteinising cysts. Because human chorionic gonadotrophin is important in the pathogenesis of neonatal ovarian cysts, it is logical to expect the cysts to regress spontaneously in the neonatal period as the hormone concentrations fall and the stimulus for growth disappears. This often seems to happen unless some complication supervenes.[8 12] All the reported cases of spontaneous resolution have occurred within four months of birth.

Both sides seem to be affected with equal frequency, also a finding in our series. Bilateral cysts were said to be rare, only three cases having been described by 1974,[12] though the incidence has increased since the introduction of ultrasonography; three of our cases had bilateral cysts.

Malignant change in neonatal ovarian cysts that are simple fluid filled structures is extremely rare, and is usually seen only in more complex lesions.[1] Other complications, however, are common. Most commonly reported complications of untreated ovarian cysts are torsion of the pedicle (cases 3, 4, 5, and 7), haemorrhage into the cyst or abdominal cavity, and rupture of the cyst.[1 3 5 6 15]

The cysts may be of any size, the largest reported being over 20 cm in diameter. The larger cysts are associated with other complications, including intestinal obstruction with one reported case of caecal perforation in an infant,[16] and hydramnios. One of our cases was associated with hydramnios (case 7) and another probably had intermittent intestinal obstruction (case 5). Hydramnios has been reported in 5 to 10% of pregnancies in which a fetal ovarian cyst was subsequently diagnosed.[12 17 18 19] This is probably caused by pressure of the mass on the small bowel together with interference with the swallowing mechanism of the fetus, which reduces ingestion and absorption of amniotic fluid.

There is a risk of pulmonary hypoplasia developing in fetuses with large cysts.[7] Pulmonary hypoplasia is associated with other lesions which compress and reduce the intrathoracic space (for example, large abdominal fluid collections and masses, and diaphragmatic hernias and eventration). Though there have been no definite cases reported of pulmonary hypoplasia developing secondary to an ovarian cyst, the risk exists if the cyst is large enough to cause thoracic compression as with any other large abdominal mass.

Nowadays the diagnosis of ovarian cysts is by ultrasound scan and may be made antenatally or postnatally. Mesenteric and duplication cysts may, however, be ultrasonically indistinguishable from ovarian cysts.[6] Most neonatal ovarian cysts are asymptomatic and found incidentally. Some of the cysts are extremely mobile and appear as a 'wandering' tumour (as seen in cases 3 and 6.)[5]

The treatment of these ovarian cysts is controversial. Until recently the recommended treatment was surgical. If the cyst was unilateral or if there had been torsion, oophorectomy was performed. The rationale for this when a simple unilateral cyst was present was the risk of possible torsion, or the replacement of the entire ovary by cyst making cystectomy impossible, or the inability to distinguish cyst from remaining normal but distorted ovarian tissue on histological examination.[3 4 6] More recently simple cystectomy was recommended but whether any function remained in the ovary is unknown.[6] The cystectomy specimens in our series all contained normal ovarian tissue, thus supporting the above findings. This is not of any great importance if there is a normal ovary on the other side.

Problems arise, however, when the cysts are bilateral. Oophorectomy on one side and cystectomy on the other have been done, as have bilateral cystectomies. These operations carry a high risk of removing all normal ovarian tissue (despite attempts to preserve some of each ovary), thereby rendering the patient sterile.

All the cases in our series had either cystectomy or salpingo-oophorectomy. We therefore decided to see whether alternative and more conservative treatment had been reported, and, indeed surgical conservatism had been advocated for some time and the advent of cyst aspiration was predicted in 1972.[3]

In a recent paper, Nussbaum *et al* described three cases in which cysts had resolved spontaneously within four months of discovery.[8] All were less than 5 cm in diameter and were followed up by serial ultrasound scans. Though there is a potential risk of torsion, only two cases have been reported in cysts of less than 5 cm in diameter.[5 6] In both cases the torsion was detected on the ultrasound scan because the previously echo free cyst developed fluid and debris levels within it. Both cases underwent operations, though it is not clear whether there is a risk in leaving a twisted, infarcted cyst untreated. Rupture of the cyst is rare and has only been reported in larger lesions (10 to 12 cm in diameter); it would not therefore seem to be a risk factor in lesions less than 5 cm in diameter.[15]

Lesions more than 5 cm in diameter present a greater problem as there is an increased risk of complications if they are left untreated. In view of the natural tendency of these cysts to regress spontaneously we suggest that cyst puncture should be performed, and the patient followed up with serial ultrasound scans. Recurrence of the cyst could be treated by repeated aspiration, surgical removal being reserved for the few intractable or complicated cases. There have been two recent case reports of intrauterine ovarian cyst aspiration in which there was thought to be an appreciable risk of pulmonary hypoplasia secondary to a pronounced degree of thoracic compression.[7] The most important risk associated with cyst puncture is cyst rupture leading to peritonitis. This is, however, uncommon.[20]

Oestradiol concentrations should be measured in aspirated fluid to confirm its ovarian origin,[21] though high levels of oestrogen are found in the normal neonatal circulation.[22] This oestrogen, the exact structure of which is unknown, is probably manufactured and secreted by the fetal adrenal glands and may interfere with the oestradiol estimation by some of the direct assay kits used in some laboratories. Spuriously high levels of oestradiol may, therefore, be detected in the asipirate if the sample is contaminated with blood. This should be borne in mind if the laboratory uses these kits.[23] A high oestradiol concentration in clear aspirate from a cyst indicates with certainty that it is of ovarian origin.

A conservation approach to this problem should therefore be attempted before embarking on surgical treatment. The advantages of this are firstly, that the patient may require no treatment if the cyst regresses spontaneously on serial ultrasound scans. Secondly, if cyst aspiration is effective then the patient is spared an operation, and thirdly—perhaps most importantly—if the cysts are bilateral the conservative approach prevents the possible removal of all normal ovarian tissue. We therefore propose therapeutic regimens for the treatment of uncomplicated neonatal ovarian cysts (tables 1 and 2). Complicated cysts should be treated with as conservative an operation as possible.

We thank Mr RE Cudmore for permission to report some of the cases that were under his care, and Mrs J Scott for typing the manuscript.

Table 1 *Regimen for antenatal diagnosis of uncomplicated neonatal ovarian cysts*

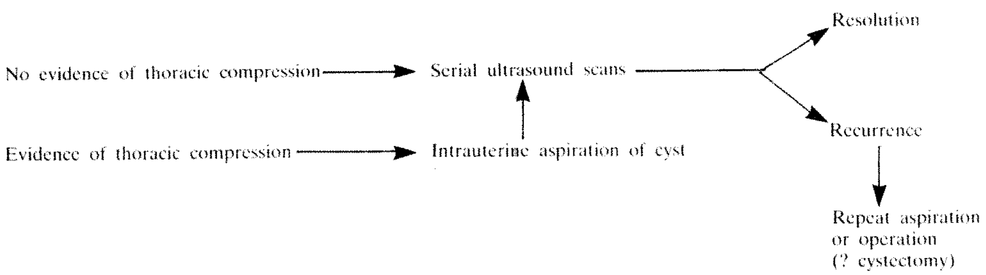
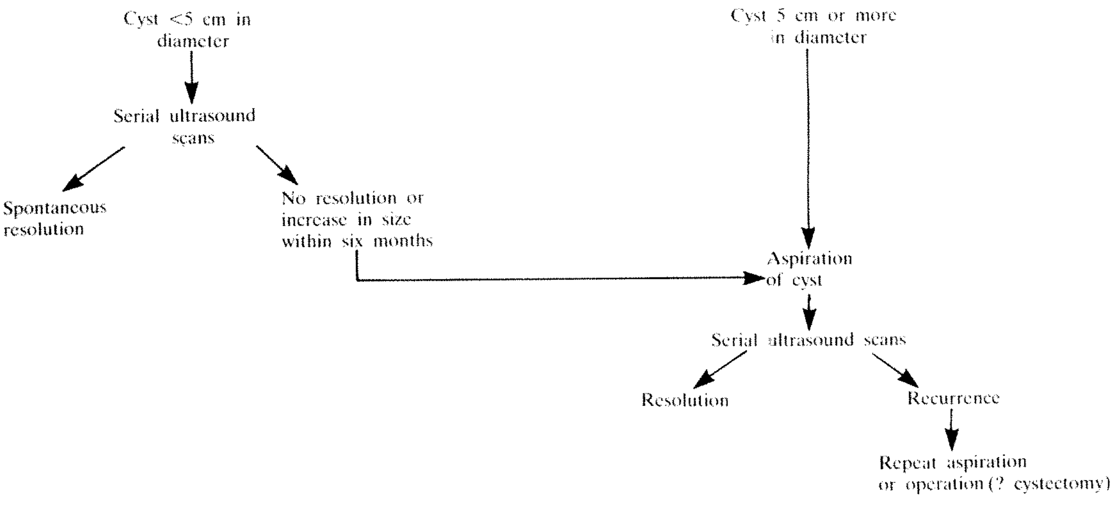

No evidence of thoracic compression
Serial ultrasound scans
Resolution
Recurrence
Evidence of thoracic compression
Intrauterine aspiration of cyst
Repeat aspiration or operation (? cystectomy)

Table 2 *Regimen for postnatal diagnosis of uncomplicated neonatal ovarian cysts*



References

[1] Marshall JR. Ovarian enlargements in the first year of life: Review of 45 cases. *Ann Surg* 1965;**161:**372–7.

[2] Ahmed S. Neonatal and childhood ovarian cysts. *J Pediatr Surg* 1971;**6:**702–8.

[3] Carlson DH. Griscon NT. Ovarian cysts in the newborn. *American Journal of Roentgenology, Radium Therapy and Nuclear Medicine* 1972;**116:**664–72.

[4] Alvear DT. Rayfield MM. Bilateral ovarian cysts in early infancy. *J Pediatr Surg* 1976;**11:**993–5.

[5] Avni EF, Godart S. Israel C. Schmitz C. Ovarian torsion cyst presenting as a wandering tumor in a newborn: antenatal diagnosis and postnatal assessment. *Pediatr Radiol* 1983;**13:** 169–71.

[6] Montagne JPH. Neuenschwander S. Prot D. Cordier MD. Solitary intraperitoneal cystic lesions in newborn girls: ten cases studied by ultrasound. *Ann Radiol* 1982;**25:**131–5.

[7] Landrum B, Ogburn PL. Feinberg S. *et al.* Intrauterine aspiration of a large fetal ovarian cyst. *Obstet Gynecol* 1986;**68** (suppl 3):11–14.

[8] Nussbaum AR. Sanders RC. Benator RM. Haller JA. Dudgeon DL. Spontaneous resolution of neonatal ovarian cysts. *AJR* 1987;**148:**175–6.

[9] Kraus FT. Neubecker RD. Luteinisation of the ovarian theca in infants and children. *Am J Clin Pathol* 1962;**37:**389–97.

[10] Curtis EM. Normal ovarian histology in infancy and childhood. *Obstet Gynecol* 1962;**19:**444–54.

[11] De Sa DJ. Follicular ovarian cysts in stillbirths and neonates. *Arch Dis Child* 1975;**50:**45–50.

[12] Bower R. Dehner LP. Ternber JL. Bilateral ovarian cysts in the newborn. A triad of neonatal abdominal masses, polyhydramnios and maternal diabetes. *Am J Dis Child* 1974;**128:**731–3.

[13] Ahlvis RC. Bauer WC. Luteinised cysts in the ovaries of infants born of diabetic mothers. *Am J Dis Child* 1957;**93:**107–9.

[14] Sedin G. Berquist C. Lindren PG. Ovarian hyperstimulation syndrome in preterm infants. *Pediatr Res* 1985;**19:**548–52.

[15] Manson R. Rodgers BM. Nelson RM. Young TK. Ruptured ovarian cyst in a new born infant. *J Pediatr* 1978;**73:**324–5.

[16] Schotz PM. Key L. Filston HC. Large ovarian cyst causing cecal perforation in a new born infant. *J Pediatr Surg* 1982;**17:**91–2.

[17] Kramer EE. Hydramnios, oligohydramnios and fetal malformations. *Clin Obstet Gynecol* 1966;**9:**508–19.

[18] Naeye RL, Milic AMB. Blanc W. Fetal endocrine and renal disorders. Clues to the origin of polyhydramnios. *Am J Obstet Gynecol* 1970;**108:**1251–6.

[19] Neikirk WI, Hudson HW Jr. Giant ovarian cyst in newborn infant. *N Engl J Med* 1948;**239:**468–9.

[20] Valenti C, Kassner EG. Yermakov et al. Antenatal diagnosis of fetal ovarian cyst. *Am J Obstet Gynecol* 1975;**123:**216–9.

[21] Montag TW. Auletta FJ. Gibson M. Neonatal ovarian cyst: Prenatal diagnosis and analysis of the cyst fluid. *Obstet Gynecol* 1983;**61**(suppl 3):385–415.

[22] Winter JSD. Hughes IA. Reyes FI *et al.* Pituitary-gonad relationships in infancy: 2. Patterns of serum gonadal steroid concentrations in man from birth to 2 years of age. *J Clin Endocrinol Metab* 1976;**42:**679–85.

[23] Diver MJ. Nisbet JA. Warning on plasma oestradiol measurement. *Lancet* 1987;**ii:**1097.

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Comparison of capillary and arterial blood gas measurements in neonates

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SUMMARY One hundred and fifty eight paired arterial and capillary blood samples were obtained from 41 sick preterm infants in their first week of life and the pH, and PCO_2, and PO_2 concentrations were measured. Half of the capillary samples were obtained from unwarmed heels and half from heels warmed to 40°C. A potentially significant discrepancy (arbitrarily defined as 0·05 units for pH, 1 kPa for PCO_2, and 3 kPa for PO_2) was found in 19 (24%) of cases for pH, in 9 (11%) for PCO_2 and in 21 (26%) for PO_2. Warming the heel produced no significant improvement in results. We conclude that capillary blood provides satisfactory measurements of pH and PCO_2 for all but the most critical purposes, but that the usefulness of capillary PO_2 estimations is limited to the exclusion of hypoxia.

Blood gas analysis is often performed for neonates on blood samples taken either from arteries or capillaries, but there have been few publications comparing the results obtained from these two sources in babies. Studies by Gandy *et al* in babies weighing over 2000 g at birth,[1] and by Koch *et al*,[2] and MacRae *et al*[3] in healthy term babies, showed that the pH and PCO_2 measurements of arterial and capillary blood collected from warmed heels were similar after the first few hours of life. Arterial and capillary PO_2 measurements showed a less strong association. Gandy *et al*[1] found a poor correlation, and Glascow *et al*[4] and Usher[5] found that the correlation was reasonable only when the arterial PO_2 was below 8 kPa. Winquist *et al*,[6] using histamine iontophoresis to arterialise capillary blood, showed a close correlation in preterm babies over 5 days old, and Corbett *et al*[7] and Desai *et al*[8] described a good correlation between arterial and capillary PO_2 using the dorsal surfaces of the finger and the scalp, respectively, as the sources of capillary blood.

In only one of the publications cited[6] were ill preterm babies studied; and that was in 1970 when babies in the neonatal intensive care unit were considerably more mature than they are today. We therefore felt that an up to date investigation of the association between arterial and capillary blood gas measurements was warranted.

A questionnaire sent to 42 neonatal units showed that more than half relied on capillary samples for most of their blood gas estimations. One third of the units attempted to warm the site from which the blood was taken before sampling. Twenty nine of the respondents thought that capillary PO_2 measurement was of no value, 11 thought that it was reliably 2–3 kPa below the arterial value, and two thought that it accurately reflected the arterial PO_2. The present study was designed to compare capillary blood gases taken from unwarmed heels and from heels warmed to 40°C with arterial gases to find out whether the capillary measurements were sufficiently accurate to be used in clinical practice.

Patients and methods

One hundred and fifty eight paired arterial and capillary blood samples were obtained from 41 preterm babies, median gestational age 30 weeks (range 23–34). All were between 3 hours and 7 days old and had indwelling umbilical arterial catheters. At the time of sampling each baby had a normal central temperature (36·8°–37·2°C) and a mean arterial blood pressure above 30 mm Hg. Thirty five of the babies had respiratory distress syndrome, and 57% were receiving muscle relaxants at the time of sampling. All capillary samples were obtained when capillary puncture was performed for Dextrostix measurement—this is routine practice as our arterial lines are perfused with dextrose. The study was approved by the local ethical committee.

The first pair of samples from each infant comprised an arterial sample and a capillary sample taken simultaneously from an unwarmed heel (group 1). The second pair comprised an arterial sample and a capillary sample taken simultaneously from a heel warmed in water at 40°C for five minutes before sampling (group 2). The capillary samples were taken from the medial and lateral borders of the heel using an Autolet; a 200 μl sample of blood was collected into a heparinised tube. Arterial samples were collected after a 2 ml 'dead space' volume had been removed from the line. All samples were analysed on a Radiometer ABL 300 blood gas analyser within five minutes of being taken. The machine's precision was tested by analysing 10 consecutive samples from the same source. The standard deviations for pH, PCO_2 and PO_2 were 0·001, 0·04 kPa, and 0·3 kPa, respectively.

Confidence intervals are reported for the data that approximated to a normal distribution. The results for PO_2 have a skewed distribution and were analysed by Kendall's S test, a non-parametric, distribution free method.

Results

Seventy nine paired samples were obtained for each group. For each of the measured variables we studied the distribution of arterial and capillary measurements, the distribution of the differences between paired arterial and capillary measurements (referred to as 'discrepancies'), and the proportion of results in which it was thought that the discrepancy was of potential clinical importance, (0·05 units of pH, 1 kPa for PCO_2, and 1, 2, and 3 kPa for PO_2).

The distribution of pH measurements is shown in fig 1 and the discrepancies between arterial and capillary measurements in table 1. There was no significant difference in mean discrepancy between the two groups (95% confidence intervals −0·006 to +0·014 units; the 5% significance figure of +0·004 lies within this interval). This suggests that warming the heel had little beneficial effect on pH measurement. Overall, 28 of 158 (18%) of the paired samples were discrepant by more than 0·05 units of pH (that is, about a 12% difference in hydrogen ion concentration).

Table 1 *Discrepancy between arterial and capillary pH measurements in paired samples taken from unwarmed heels (group 1) and warmed heels (group 2)*

	Group 1 *(n=79)*	*Group 2* *(n=79)*
Mean (SD) discrepancy (arterial minus capillary value)	0·005 (0·035)	0·001 (0·033)
95% confidence intervals	−0·06 to 0·07	−0·06 to 0·07
No (%) of results in which discrepancy exceeded 0·05 units	19 (24)	9 (11)

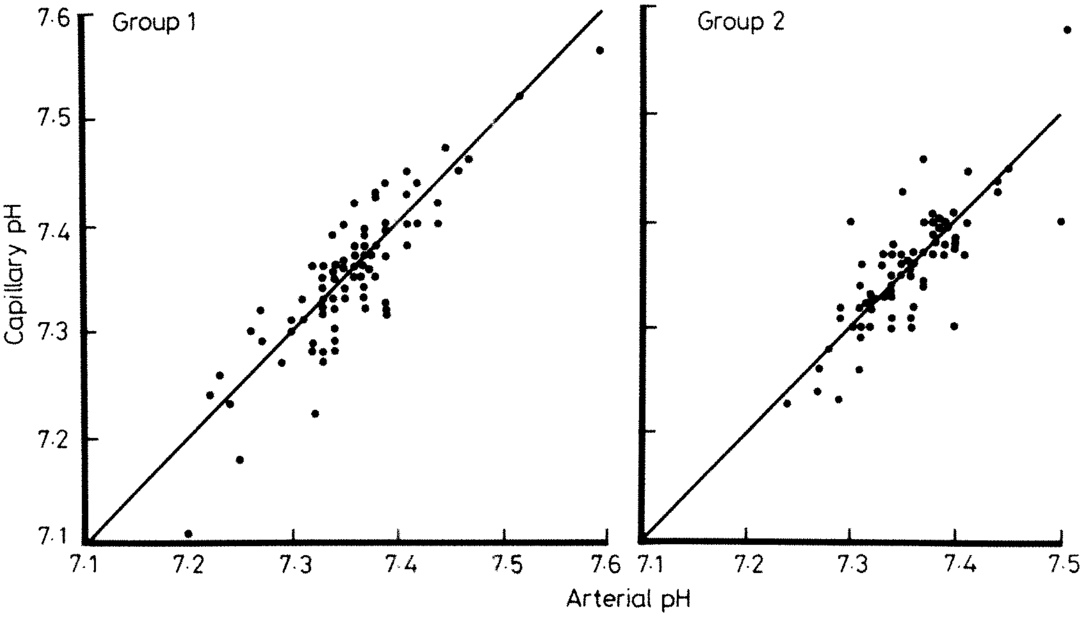

Fig 1 *pH of arterial and capillary blood in paired samples taken from unwarmed heels (group 1) and warmed heels (group 2) with line of identity.*

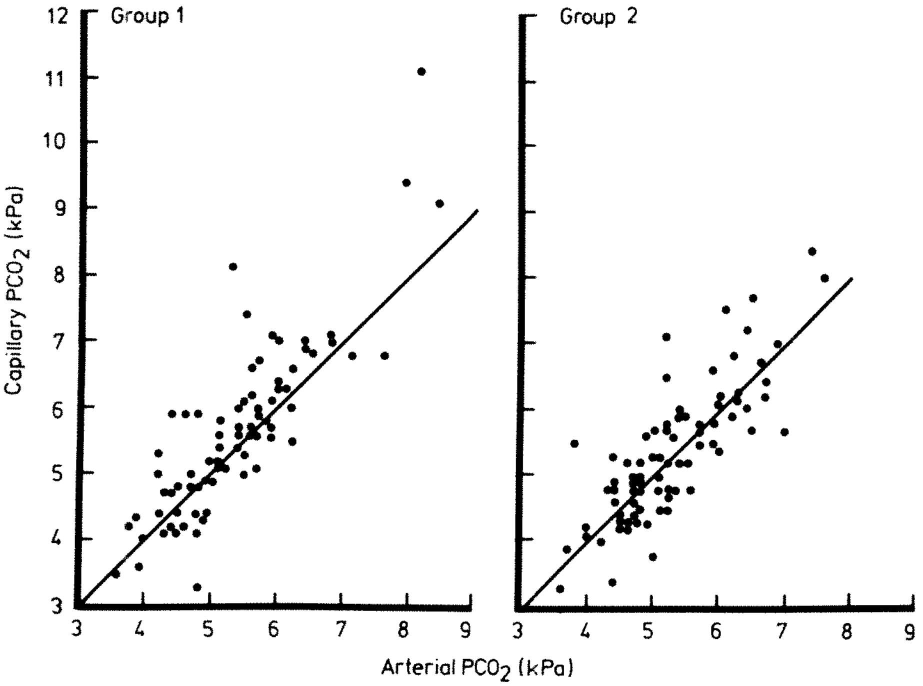

Fig 2 *PCO_2 of arterial and capillary blood in paired samples taken from unwarmed heels (group 1) and warmed heels (group 2) with line of identity.*

The distribution of PCO_2 measurements is shown in fig 2 and the discrepancies between arterial and capillary measurements are shown in table 2. The difference in the mean discrepancy between the two groups was not significant at the 5% level (95% confidence intervals −1·1 to +1·5 kPa; the 5% significance figure of −0·21 lies within this interval), indicating that warming the heel had little effect. Overall, 15 of 158 (10%) of the paired samples were discrepant by more than 1 kPa.

The distribution of PO_2 measurements is shown in fig 3, and the discrepancies between arterial and capillary measurements in table 3 and fig 4. A comparison of the discrepancies between the two groups showed no significant difference at the 5% level, again suggesting that warming the heel made little difference (S=1036; variance(s)=3944 and 312; SD 1·8; $p>0·1$).

Table 2 *Discrepancy between arterial and capillary PCO_2 measurements in paired samples taken from unwarmed heels (group 1) and warmed heels (group 2)*

	Group 1 (n=79)	*Group 2 (n=79)*
Mean (SD) discrepancy (arterial minus capillary value)	−0·26 kPa (0·7)	−0·05 kPa (0·65)
95% confidence intervals	−0·42 to +0·1 kPa	−0·18 to +0·08 kPa
No (%) of results in which discrepancy exceeded 1 kPa	9 (11)	6 (8)

Discussion

The merits of sampling capillary blood for analysis of blood gases are that it is technically easier and less likely to result in serious complications than arterial catheterisation or puncture, and that sites are available when arterial sites are not, but it is a clinically useful technique only if the blood gas measurements obtained are a sufficiently accurate guide to the arterial measurements. In this study the arterial blood gas measurements were taken as the 'gold standard' by which capillary measurements were judged. To what extent the discrepancies reported are methodological (for example, diffusion of CO_2 from capillary blood during sampling), or physiological is uncertain, but a standard capillary blood sampling procedure was used and more attention was probably paid to good technique than can usually be guaranteed, and so the study is

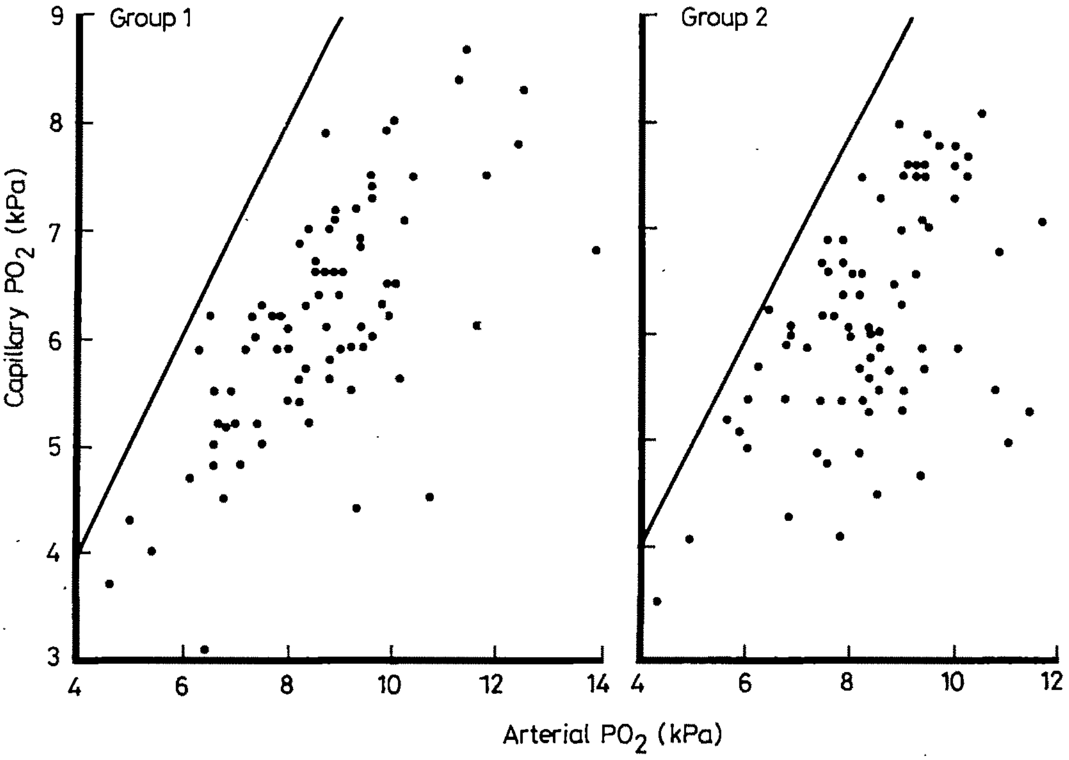

Fig 3 *PO_2 of arterial and capillary blood in paired samples taken from unwarmed heels (group 1) and warmed heels (group 2) with line of identity.*

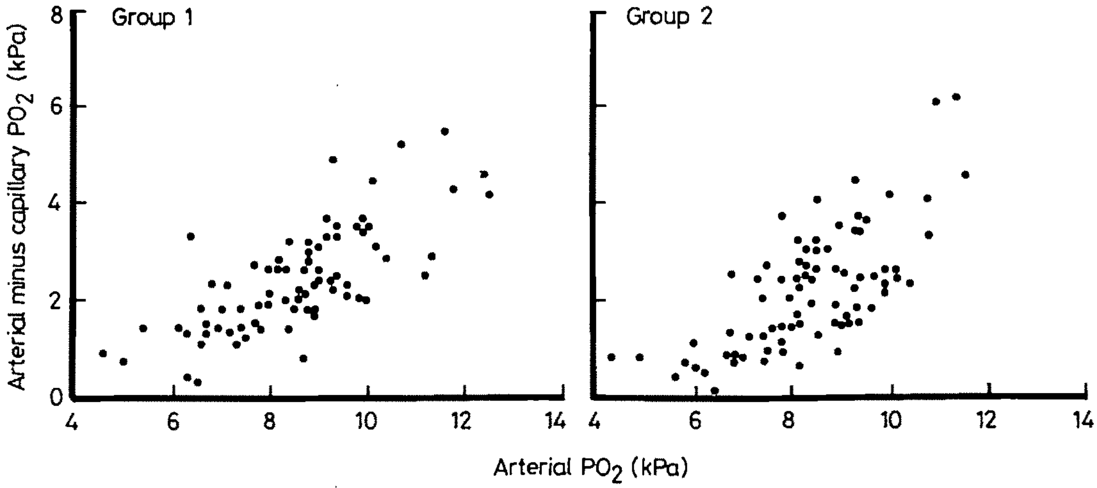

Fig 4 *Discrepancy between arterial and capillary PO_2 measurements in samples taken from unwarmed heels (group 1) and warmed heels (group 2) and arterial PO_2 measurements in the two groups.*

Table 3 *Discrepancy between arterial and capillary PO_2 measurements in paired samples taken from unwarmed heels (group 1) and warmed heels (group 2)*

	Group 1 *(n=79)*	*Group 2* *(n=79)*
Mean (SD) discrepancy (arterial minus capillary value)	2·47 kPa (1·20)	2·17 kPa (1·25)
Range	0·3 to 7·2 kPa	0·1 to 6·1 kPa
No (%) of results in which discrepancy exceeded:		
1 kPa	74 (94)	59 (75)
2 kPa	47 (59)	42 (53)
3 kPa	21 (27)	16 (20)

representative of what happens in practice. It is unlikely that a consensus could be reached among paediatricians as to what is a 'sufficiently accurate' guide to arterial measurements, and undoubtedly this also depends on the state of the patient. From the results of this study we contend that in acutely ill preterm babies, for whom no effort is spared to maintain meticulous homoeostasis, capillary blood analysis may not reflect any arterial blood gas variable with sufficient accuracy.

In less critical circumstances the measurements of pH and PCO_2 derived from capillary blood were satisfactory, but there is limited clinical usefulness in the association between arterial and capillary PO_2 measurements. The tendency for the discrepancy in PO_2 to diminish as lower figures were approached, and the fact that capillary PO_2 never exceeded arterial PO_2, confers some value on capillary blood gas analysis as a means of excluding hypoxia. Hyperoxia, on the other hand, is difficult to exclude by capillary blood gas analysis. It is tempting to interpret fig 3 as suggesting that arterial PO_2 measurements above 11 kPa are unlikely as long as the capillary PO_2 is kept below about 6 kPa, but this is to extrapolate beyond the limits of the data as there are not enough babies with excessively high arterial PO_2 measurements to support this conclusion.

In this study warming the heel made no significant difference to the discrepancy between arterial and capillary measurements of any of the variables. It may be that temperatures above 40°C would be more effective in arterialising capillary blood, but we were concerned about the possibility of damaging the skin.

References

[1] Gandy G, Grann L, Cunningham N. The validity of pH and pCO_2 measurements in sick and healthy newborn infants. *Pediatrics* 1964;**34:**192–7.

[2] Koch G, Wendel H. Comparison of pH, carbon dioxide tension, standard bicarbonate and oxygen tension in arterial and capillary blood in the newborn period. *Acta Paediatr Scand* 1967;**56:** 10–16.

[3] MacRae OJ, Pulavradji O. Comparison between arterial, capillary and venous acid base measurements in the newborn infant. *Journal of Obstetrics and Gynaecology of the British Commonwealth* 1966;**75:**761–5.

[4] Glascow JFT, Flynn DM, Swyer PR. A comparison of descending aortic and arterialised capillary blood in the sick neonate. *Can Med Assoc J* 1972;**106:**660–3.

[5] Usher R. Discussion of complications arising from catheterisation of umbilical vessels. In: Lucey JF, ed. *Problems of neonatal intensive care. A report of the 59th Ross Conference of Paediatric Research.* New York: Columbus Press, 1969:36–43.

[6] Winquist RA, Stamm SJ. Arterialised capillary sampling using histamine iontophoresis. *J Pediatr* 1970;**76:**455–8.

[7] Corbett AJS, Burnard ED. Oxygen tension measurements on digital blood in the newborn. *Pediatrics* 1970;**46:**780–2.

[8] Desai JD, Holloway R, Thambiran AK. A comparison between arterial and arterialised capillary blood in infant. *S Afr Med J* 1967;**41:**13–15.

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Short reports

Serum antioxidant activity in neonates

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SUMMARY Antioxidant activity was measured in cord sera from 25 infants whose birth weights ranged from 830 to 3700 g. Antioxidant activity showed a significant positive association with birth weight. Deficiency in serum antioxidant activity may be important in the pathogenesis of certain neonatal disorders such as retinopathy of prematurity.

The plasma proteins ceruloplasmin and apotransferrin have strong antioxidant activities, as measured by their ability to prevent autoxidation in brain homogenates.[1] The antioxidant activity of both proteins involves interactions with iron; the antioxidant activity of apotransferrin depends on its ability to bind two ferric ions and that of ceruloplasmin depends on its ferroxidase activity. The antioxidant potency of these two proteins in the brain homogenate assay has been estimated to be 200–500 times that of α-tocopherol.[1]

In babies born at term the plasma concentrations of ceruloplasmin and apotransferrin are low by adult standards, and they are even lower in premature infants.[2 3] These findings imply that serum antioxidant activity is low in premature infants and increases with gestational age. Such a pattern might help to explain the apparently increased susceptibility of some premature infants to the adverse effects of increased oxygen tensions.

Methods

Antioxidant activity was measured in cord sera from 25 infants by a modification of the method of Stocks *et al.*[1] Birth weights ranged from 830 to 3700 g. Antioxidant activity was also measured in sera from a group of 21 adult blood donors, 12 men and nine women with a mean age of 31·5 years.

To measure serum antioxidant activity, 40 μl of serum was added to 2 ml of ice cold bovine brain homogenate (about 1:10 w/v in phosphate buffered saline, pH 7·4). The mixture of serum and homogenate was then serially diluted with ice cold homogenate and incubated at 37°C for one hour. Thiobarbituric acid reacting substances generated by spontaneous autoxidation of the brain homogenate were determined by measuring the absorbance in the supernatants at 535 nm after addition of 2 ml of thiobarbituric acid reagent mixture, heating, and centrifugation. Controls contained either homogenate alone or homogenate with butylated hydroxytoluene at a final concentration of 60 μM. Residual autoxidation was calculated by dividing the absorbance at 535 nm of the cord serum with homogenate (minus the absorbance at 535 nm, of the homogenate with butylated hydroxytoluene) by the absorbance at 535 nm of the homogenate control (minus the absorbance at 535 nm of the homogenate with butylated hydroxytoluene). Residual autoxidation was plotted against the amount of serum present in six serial twofold dilutions. The x intercept of the resulting straight line, derived by linear regression, was the serum dose in μl (Dmax) required for maximal inhibition of autoxidation. A large value for Dmax indicates a small antioxidant activity.

A wide range of serum doses in each case was used to give a better estimate of extreme values of antioxidant activity. Use of a single serum dose, and expression of antioxidant activity as per cent inhibition of the formation of thiobarbituric acid reacting substances would compress the range of observed antioxidant activities.[1] Antioxidant activity, defined as per cent inhibition, is proportional to reciprocal Dmax.

To show the corrective effects of various substances on antioxidant activity, 20 μl samples of a selected infant's cord serum were added to 5 μl of selected adult sera, or to 5 nmol of d-penicillamine, dl-α-tocopherol, deferoxamine mesylate, or human apotransferrin. The infant weighed 1480 g and had extremely low antioxidant activity with an indeterminately large Dmax. The final concentration of each substance was 125 μM in the resulting mixture, before the addition of homogenate. Triplicate 1 ml samples of the mixtures were incubated with homogenate for 1 hour at 37°C. Formation of thiobarbituric acid reacting substances was expressed as net absorbance at 535 nm after subtraction of the absorbance at 535 nm in homogenates treated with butylated hydroxytoluene; dl-α-tocopherol was added in ethanol. Correction for the effect of ethanol was not made because the ethanol control was not significantly different from the infant serum alone (unpublished). The adult sera were from a 34 year old man who had not donated blood and a 20 year old woman who was a frequent blood donor.

Results

In this group of 25 infants, Dmax was inversely related to birth weight (fig 1) and this was significant by Spearman's rank correlation ($p<0·05$). A previous study found that antioxidant activity in infants born at term measured as per cent inhibition of formation of thiobarbituric acid reacting substances was only about 20% of the normal adult value.[4] In the present study the 12 infants who weighed more than 2500 g at birth had a mean (SEM) Dmax of 41·1 μl (4·52 μl) compared with a mean (SEM) for the adult donor group of 12·2 μl (0·955 μl), ($p<0·001$). These findings are comparable with those previously reported.[4]

The ability of various substances to correct the deficient antioxidant activity of one infant's serum was investigated, d-penicillamine, dl-α-tocopherol, deferoxamine mesylate, and human apotransferrin being added in eqimolar amounts to give final concentrations that roughly approximated to those that are achievable in an infant's plasma. The dl-α-tocopherol concentration was near the upper limit achieved by pharmacological doses in a recent study of retinopathy of prematurity.[5]

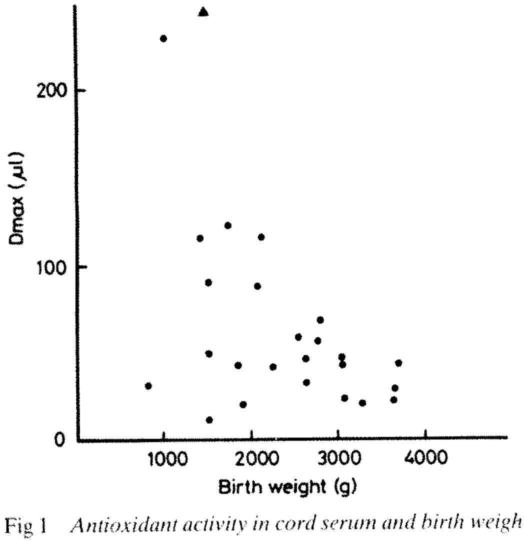

Fig 1 *Antioxidant activity in cord serum and birth weight in 25 infants.*

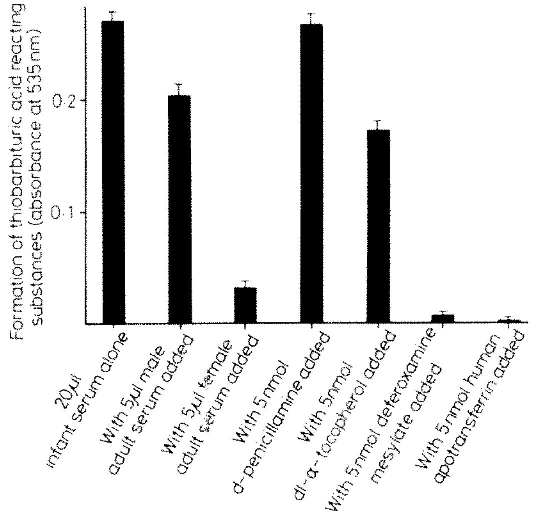

Fig 2 *Effects of addition of adult sera, penicillamine, dl-α-tocopherol, deferoxamine, and human apotransferrin on antioxidant activity of cord serum from an infant weighing 1480 g. (Figures are means of three determinations).*

As shown in fig 2, dl-α-tocopherol, deferoxamine mesylate, human apotransferrin and adult sera were able to decrease thiobarbituric acid reacting substances formation significantly. Deferoxamine mesylate and human apotransferrin were significantly superior ($p<0·001$) to dl-α-tocopherol in decreasing thiobarbituric acid reacting substances formation. The addition of α-pencillamine did not result in a significant decrease. Human apotransferrin, deferoxamine mesylate, and the adult female serum were by far the most effective at correcting the infant's antioxidant activity.

Discussion

These data confirm the report of Gutteridge and Stocks that antioxidant activity in the serum of term neonates is lower than that of adults.[4] The present study extends the applicability of their findings to premature infants. Antioxidant activity in the lower birthweight infants was generally lower than that in high birthweight infants. In some cases at the lower end of the birthweight range, extremely high values for Dmax were found. This pattern is comparable with the low concentrations of human apotransferrin and ceruloplasmin in premature infants found by other workers.[2 3]

The possible antioxidant role of plasma dl-α-tocopherol in neonatal disorders such as retinopathy of prematurity has received much attention.[5] The present findings raise the possibility that deficiencies in the potent human apotransferrin/ceruloplasmin dependent antioxidant system may be important in the pathogenesis of retinopathy of prematurity and similar disorders.

These findings are relevant to the suggestion[6] that increased plasma concentrations of non-haem iron in the neonatal period are of central importance in the pathogenesis of retinopathy of prematurity. Large increases in serum ferritin and transferrin iron saturation are usually seen in premature infants and infants born at full term shortly after birth. Additional sources of exogenous iron in the neonatal period include dietary iron supplementation and blood transfusion.[6] The present data suggest that one important effect of increased non-haem iron may be to neutralise much of the premature infant's scarce antioxidant activity, thereby sharply increasing vulnerability to oxygen.

We thank Dr DL Phelps and Dr WA Silverman for their helpful suggestions, Dr DM Purohit for help in obtaining samples of cord serum, and American Red Cross Blood Services, Carolina Low Country Region for samples of serum from blood donors. RBN had a postdoctoral fellowship from the College of Graduate Studies of the Medical University of South Carolina, and the work was supported by the Veterans Administration.

References

[1] Stocks J, Gutteridge JMC, Sharp RJ, Dormandy TL. The inhibition of lipid autoxidation by human serum and its relation to serum proteins and α-tocopherol. *Clinical Science and Molecular Medicine* 1974;47:223–33.

[2] Hilderbrand DC, Fahim Z, James E, Fahim M. Ceruloplasmin and alkaline phosphatase levels in cord serum of term, preterm and physiologically jaundiced neonates. *Am J Obstet Gynecol* 1974;118:950–4.

[3] Scott PH, Berger HM, Kenward C, Scott P, Wharton BA. Effect of gestational age and intrauterine nutrition on plasma transferrin and iron in the newborn. *Arch Dis Child* 1975;**50:** 796–8.

[4] Gutteridge JMC, Stocks J. Caeruloplasmin: physiological and pathological perspectives. *CRC Crit Rev Clin Lab Sci* 1981;**14:** 257–329.

[5] Phelps DL, Rosenbaum AL, Isenberg SJ, Leake RD, Dorey FJ. Tocopherol efficacy and safety for preventing retinopathy of prematurity: a randomized, controlled, double-masked trial. *Pediatrics* 1987;79:489–500.

[6] Sullivan JL. Retinopathy of prematurity and iron: a modification of the oxygen hypothesis. *Pediatrics* 1986;78:1171–2.

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Simple method for securing umbilical catheters

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SUMMARY A method for securing umbilical catheters is described. It has been used successfully in over 350 babies. It is a simple and rapid method that allows for easy repositioning and, unlike some established techniques, has no potential for damage to the preterm baby's skin.

Since their introduction in the early 1960s the techniques of umbilical arterial and venous catheterisation have been accepted into routine clinical practice. Adequate fixation of catheters is important: displacement can lead to malposition of the catheter tip, with consequent poor sampling and increased risk of vascular thrombosis; catastrophic haemorrhage can occur if the catheter 'falls out.'

Most recommended methods for catheter fixation involve a combination of typing loops of suture material around the base of the catheter, and the use of a 'goalpost' bridge of adhesive tape attached to the catheter and to the skin of the abdominal wall.[1] Having used such methods for a number of years we became aware of several problems: tying the loops of suture material is fiddly, and it can be difficult to tie them sufficiently tight to grip the catheter without partially occluding its lumen. The adhesive tape is often difficult to attach to the baby's moist, vernix covered skin. The tape reduces the area available for the use of transcutaneous electrodes. The bridge of tape can be caught accidentally during handling, and it has even provided an effective footstrap for an active baby to kick against. Removal of adhesive tape from the often extremely fragile skin of a very preterm baby can be damaging and can lead to ooze of plasma, increased fluid loss, and risk of sepsis. Once fixation is complete it can be difficult to reposition the catheter if a radiograph shows an unsatisfactory placement of the tip.

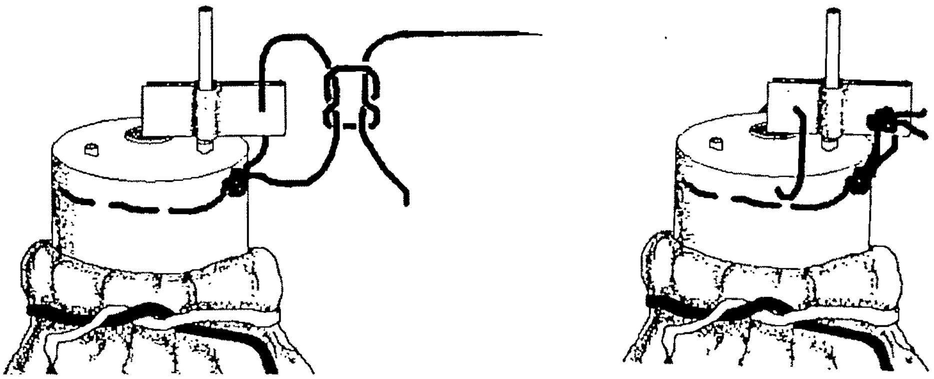

Figure *Left—purse-string suture is passed through the zinc oxide tape and tied. Right—second suture is passed through the cord stump to transfix the umbilical vein. It is then taken through the zinc oxide tape on the opposite side to the first suture and then tied.*

In an attempt to overcome some of these problems we have used a different method of fixation.

Method

When the catheter had been inserted to the required depth, a purse-string 3/0 black silk suture was placed into the cord stump, the needle was left attached to one free end. The base of the catheter was dried with a gauze swab and a piece of 1 cm wide zinc oxide tape (no other tape will do) about 2 cm long was folded around the catheter as close as possible to the cord stump. The needle of the suture was then passed through the tape close to the catheter and tied (fig—left). The needle, with a long thread attached, was then cut off close to the knot. The needle was passed through the cord stump to transfix the umbilical vein, through the zinc oxide tape on the other side of the catheter, and then tied (fig—right). No further fixation was used.

We preferred to place the tip of umbilical arterial catheters at the level of the diaphragm. If assessment by radiography showed the catheter position to be unsatisfactory, it was withdrawn either to the diaphragmatic level (for catheters placed too high) or the low (L3-4) position (for catheters placed too low). To achieve this the zinc oxide tape was cut on either side, close to the base of the catheter, leaving the two free pieces of tape attached by the sutures to the cord stump. The catheter was then withdrawn the measured distance and a fresh piece of zinc oxide tape was folded around the base of the catheter incorporating the two pieces of tape still attached to the stump.

Results

Since February 1985 more than 350 umbilical catheters have been secured using the method described. Three catheters became accidentally dislodged. The babies were over 2 weeks of age and were out of their incubators being weighed at the time. The fixation sutures and zinc oxide tape were all still firmly attached to the catheter, but the dried up cord had separated from the cutaneous stump leaving no secure fixation.

Discussion

This method of catheter fixation was found to be simpler and more rapid to apply than other methods. It does not lead to damage to the skin and allows for easy repositioning. The method is similar to that briefly described by Adams and Rudolph[2] but in our method the tape is stitched to the cord stump and we do not use any form of tape around the infant's waist.

No records were kept when previous methods were in use, however, the rate of catheter displacement has certainly fallen since the new technique was introduced. Three displacements occurred with catheters that were still in place after the normal time at which the cord separates. Although most catheters are removed before this time, we are attempting to prevent the problem by placing one of the sutures through the cutaneous part of the cord stump.

References

[1] Klauss MH, Fanaroff AA, eds. *Care of the high risk neonate.* 3rd ed. Philadelphia: WB Saunders, 1986:431.

[2] Adams JM, Rudolph AJ. Simple method for stabilising umbilical catheters. *Arch Dis Child* 1979;54:563.

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752 *Archives of Disease in Childhood,* 1988, **63**

Sources of error in neonatal blood sampling

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SUMMARY Significant underestimation of blood electrolyte concentrations may occur if less than 1 ml of blood is withdrawn from an indwelling arterial line before taking the sample for analysis. Compared with arterial samples capillary samples give significant overestimations of potassium concentration and carbon dioxide tension, and significant underestimation of oxygen tension.

Blood samples are regularly taken from sick neonates for measurement of electrolyte concentrations, blood gases, and pH. Our experience is that such samples are not collected in a uniform way. Standard reference works indicate how to site and take blood from indwelling arterial lines[1] but give no advice about the volume of fluid (flush volume) to remove before sampling in order to obtain accurate results. Blood counts may vary depending on the sampling site,[2] and we have encountered spuriously abnormal electrolyte and blood gas measurements from both capillary and arterial samples. We have therefore examined the effects of two different flush volumes and capillary sampling on measurements of electrolyte concentrations and blood gases.

Methods

We studied the results of all blood samples taken during a three month period from infants on the neonatal unit who had a peripheral or umbilical arterial line in situ. Lines were infused at 1 ml/hour with 5% glucose solution containing 1 unit/ml sodium heparin. The gestational age of the infants ranged from 26 to 42 weeks, and all were stable at the time of sampling with no clinical evidence of right to left ductal shunting. Arterial sampling started with the withdrawal of 0·5 ml of fluid (0·5 ml total flush) followed by the withdrawal of 0·2 ml for analysis. A further 0·3 ml was then withdrawn (1·0 ml total flush) and the next 0·2 ml sample taken; 0·8 ml was then withdrawn (2 ml total flush), and a final 0·2 ml sample taken. The 1·6 ml of fluid removed but not used for analysis was then returned to the patient. A 2 ml flush volume was chosen to minimise haemodynamic consequences for the infant. Evaluation of arterial lines in vitro had shown that a 2 ml flush volume resulted in sample dilution of only 0·2%.

A capillary sample of 0·2 ml, was taken immediately from the unwarmed heel at the same time as Dextrostix measurement was carried out. Parental consent was obtained and no infant underwent more than two procedures.

Each sample was analysed for pH, oxygen tension (kPa), and carbon dioxide tension (kPa) with a Corning 178 pH/blood gas analyser. Measurement (mmol/l) of plasma sodium and potassium concentrations was done with a Corning 902 Na-K analyser.

Statistical analysis was by the paired Student's *t* test.

Results

Forty three sets of samples were obtained for blood gas analysis and 40 sets for measurement of electro-

Table 1 *Mean (SD) differences in electrolyte measurements between other samples and 2 ml flush samples*

Plasma electrolyte concentrations (mmol/l)	*Type of sample* *0·5 ml flush*	*1·0 ml flush*	*Capillary*
Sodium	−8·3 (7·0)	−1·7 (2·3)	0·0 (2·2)
p Value	<0·001	<0·001	NS
Potassium	−0·41 (0·60)	−0·18 (0·49)	1·22 (0·96)
p Value	<0·001	<0·01	<0·001

Table 2 *Mean (SD) differences in pH and blood gas measurements between other samples and 2 ml flush samples*

Measurement	*Type of sample* *0·5 ml flush*	*1·0 ml flush*	*Capillary*
Blood pH	−0·014 (0·036)	−0·002 (0·015)	−0·023 (0·038)
p Value	<0·02	NS	<0·001
Oxygen tension (kPa)	0·19 (0·83)	0·02 (0·61)	−2·40 (1·54)
p Value	NS	NS	<0·001
Carbon dioxide tension (kPa)	−0·39 (0·56)	−0·05 (0·36)	0·52 (0·83)
p Value	<0·001	NS	<0·001

lyte concentrations from 34 infants. The results of the sodium and potassium measurements are shown in table 1 and those for pH and blood gas measurements in table 2.

Discussion

When sampling blood from neonates it is necessary to keep cumulative blood loss to a minimum by using small sample volumes (0·2 ml in this study), by limiting the number of samples that need to be repeated because of spuriously abnormal results, and by carefully assessing the clinical need for blood analysis before sampling.

The study confirmed our impression that sampling from arterial lines may result in erroneous results and hence possible unnecessary treatment. The underestimation of plasma sodium, potassium, and PCO_2 concentrations resulted from dilution of arterial blood with the small volume of 5% glucose solution in the arterial line. The smaller the flush volume of arterial blood, the greater the dilution. The mixing of 5% glucose solution (which has a pH of 5) with arterial blood led to the significantly lower pH concentrations at flush volumes of 0·5 ml.

Concentrations of potassium in capillary blood were higher than those in arterial blood in 38 out of 40 specimens, the mean value being more than 1 mmol higher but with a wide variation (SD 0·96). A high potassium concentration in a capillary sample should be confirmed by measuring the concentrations in a venous or arterial sample, the latter following a 2 ml flush if taken from an indwelling arterial line. There were significant differences between measurements in blood gases in capillary and arterial blood with a moderate rise in carbon dioxide tension in capillary blood and a larger reduction in oxygen tension in capillary blood (mean reduction 2·4 kPa), but the degree of clinical importance varied unpredictably among patients.

In conclusion, blood samples taken from arterial lines should be preceded by an adequate flush; we regard a 2 ml flush volume as ideal. Capillary blood samples may give erroneously high potassium concentrations even in the absence of obvious haemolysis. Unexpectedly abnormal results should be checked by measuring the concentrations in arterial or free flowing venous blood.

References

[1] Wilkinson A, Calvert S. Procedures in neonatal intensive care. In: Roberton NRC, ed. *Textbook of neonatology*. Edinburgh: Churchill Livingstone, 1986:817–35.

[2] Thurlbeck SM, McIntosh N. Preterm blood counts vary with sampling site. *Arch Dis Child* 1987;62:74–5.

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Cytomegalovirus screened blood for neonatal intensive care units

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SUMMARY Two infants were considered to have acquired cytomegalovirus (CMV) infection from blood transfusions screened for the presence of CMV antibody by a haemagglutination assay. Further samples from the same donors were tested by a latex agglutination test; six of 18 (33%) previously believed seronegative were found to be seropositive.

The risk of premature infants acquiring cytomegalovirus (CMV) infection from a blood transfusion can be reduced if blood components negative for CMV are used.[1] In this unit all premature babies receive blood screened for CMV. We describe two cases in which CMV infection occurred despite this policy, and discuss the implications for the provision of blood negative for CMV for neonatal intensive care units.

Case reports

CASE 1

A female infant was born at 29 weeks' gestation weighing 1200 g. After early problems she progressed well until on day 50 she deteriorated suddenly. Blood cultures grew a group A β haemolytic streptococcus. She responded to intravenous antibiotics and was discharged well at 3 months of age.

CMV was isolated from her urine at the time of her deterioration. Routine screening for CMV at birth was negative, and her mother was seronegative for CMV.

CASE 2

A female infant, the second of twins, was born at 25 weeks' gestation weighing 770 g. She was admitted to the neonatal unit two weeks after case 1. After a stormy early course she was stable on minimal ventilation by day 25. On day 32 she deteriorated suddenly with jaundice, hepatosplenomegaly, and thrombocytopenia, and her ventilatory requirements increased. Cerebrospinal fluid and repeated blood cultures were sterile.

CMV was isolated from her urine, tracheal aspirate, and blood and eye swabs. Routine CMV cultures taken at birth were negative. She gradually deteriorated and died aged 148 days. At necropsy CMV inclusion bodies were found in the lung and kidney.

Serum samples taken from her mother at 18 weeks' gestation and during the child's illness showed IgG but no IgM antibody to CMV. The first twin remained well and despite repeated viral cultures CMV was not isolated.

Possible sources of infection

CMV infection in a neonate may be acquired transplacentally,[2] during passage through the birth canal,[3] from breast milk,[3] from blood components,[1] or possibly by horizontal spread.[4] In order to exclude the possibility of horizontal spread from one of these infants to the other, virus isolates were typed using DNA restriction endonuclease analysis[5] and found to be dissimilar.

In case 1 the mother was seronegative and could not have been the source of infection. The child received breast milk only from her mother. In case 2 congenital CMV had been excluded. The infant received only parenteral nutrition. Infection during delivery was a possible source, although the other twin was not infected. Unfortunately it was not possible to take cervical or urine samples from the mother for viral studies.

Both affected infants had received multiple blood transfusions. The blood had been screened for the presence of CMV antibodies using a dilution modification of a haemagglutination assay (Cetus Corporation, subsequently withdrawn). As reported elsewhere,[6] concerns about consistency within batches and the availability of a more convenient assay had already lead to the introduction of a modified latex agglutination test (Becton Dickinson) as the preferred screening test by the blood transfusion centre.

Repeat blood samples from the donors whose blood had been given to these infants were tested for the presence of CMV antibody using the more sensitive latex agglutination test. Case 1 received blood from six donors, five of whom provided repeat samples; one was found to be positive for CMV by the latex agglutination test. Case 2 received 15 transfusions. Blood samples were obtained from 14 of the donors; five were seropositive by the latex agglutination test. One unit, used under the mistaken impression that it was seronegative, was found on rechecking to have been positive on the initial haemagglutination assay. A repeat sample from the donor, however, was seronegative by the latex agglutination test. This unit was transfused after the child acquired CMV infection.

Table *Comparison of CMV serology on repeat samples*

	First test (haemagglutination assay)		
	No positive	*No negative*	*Total No*
Second test (latex agglutination test):			
No positive	0	6	6
No negative	1	12	13
Total No	1	18	19

Overall, of the 18 donors found negative on the original assay, six (33%) were seropositive by the latex agglutination method (table).

Discussion

Failure of a test may be the result of inherent problems in the test or of human error. The haemagglutination assay was widely used and generally regarded as reliable and sensitive. Comparison with a radioimmunoassay, however, suggested that false negative results were occurring and this led to the introduction of the latex agglutination test.[6] Human error was the probable reason for the incorrect use of one unit after a positive (although probably false positive) test result.

The regional blood transfusion centre screens up to 200 units each day for the presence of CMV antibodies. Much of the demand for screened blood comes from neonatal units. When we checked some of the requests there seemed to be confusion among clinicians about the indications for the use of blood negative for CMV. Yeager *et al*[1] showed no appreciable morbidity associated with the use of unscreened blood in babies of more than 28 weeks' gestation nor in those born to seropositive mothers. They therefore recommended that blood negative for CMV should be used only for babies of less than 32 weeks' gestation born to seronegative mothers.

The mother of our child who died was seropositive; we believe that if the baby is extremely premature the restriction of screened blood to the offspring of seronegative mothers may not be appropriate. There is, however, no evidence that babies of more than 32 weeks' gestation benefit from the use of blood negative for CMV. Using gestational age rather than birth weight as the criterion for the use of blood screened for CMV seems reasonable, as morbidity from neonatal CMV infection is thought to be related to immaturity of the immune system.

Although the blood transfusion services are responsible for continuously monitoring the reliability of their test procedures, clinicians must take some responsibility. Firstly, laboratory evaluation of tests does not replace clinical observation. Any clinician who believes that a child has acquired CMV infection from blood products should discuss this with the blood transfusion service so that further investigation may be undertaken. Secondly, by limiting the use of specialised services such as the provision of blood screened for CMV to those cases for whom it is essential, the load on the service can be decreased and its efficiency increased.

References

[1] Yeager AS, Grumet FC, Hafleigh EB, Arvin AM, Bradley JS, Prober CG. Prevention of transfusion-acquired cytomegalovirus infections in newborn infants. *J Pediatr* 1981;**98:**281–7.

[2] Peckham CS, Chin KS, Coleman JC, Henderson K, Hurley R, Preece PM. Cytomegalovirus infection in pregnancy: preliminary findings from a prospective study. *Lancet* 1983;**i:**352–5.

[3] Stagno S, Pass RF, Dworsky ME, Alford CA Jr. Maternal cytomegalovirus infection and perinatal transmission. *Clin Obstet Gynecol* 1982;**25:**563–76.

[4] Spector SA. Transmission of cytomegalovirus among infants in hospital documented by restriction-endonuclease-digestion analysis. *Lancet* 1983;**i:**378–81.

[5] Huang ES, Kilpatrick BA, Huang YT, Pagano JS. Detection of human cytomegalovirus and analysis of strain variation. *Yale J Biol Med* 1976;**49:**29–43.

[6] Barbara JAJ, Moulsdale H, Brown S, Griffiths PD, Berry MJ, Contreras M. Modified latex agglutination test for anti-cytomegalovirus, suitable for pretransfusion screening. *J Clin Pathol* 1987;**40:**115–6.

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Reduction of head flattening in preterm infants

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SUMMARY During the first few weeks of life many preterm infants develop flattened heads. We have shown that this deformity can be reduced by nursing preterm infants on soft, air filled mattresses of the type used for detecting apnoea.

The shape of a newborn infant's head is independent of gestation because the support given by amniotic fluid is uniform and allows unrestrained growth.[1] After birth, however, more localised pressures are encountered and side to side flattening of the head may occur because infants are usually nursed with their heads turned laterally.[1 2] In infants born at term the effect is mild, but in preterm infants the deformity is often pronounced and may persist into adulthood.[1] This is probably because preterm infants have thin, soft skull bones that are more malleable. In addition, they spend many weeks lying only on the sides of the head until they become developmentally mature enough to adjust their position voluntarily.

Head flattening has two clinical consequences. Firstly, it may result in misleadingly high measurements of occipitofrontal circumference because for a given area the perimeter of an ellipse is greater than that of a circle.[1] Secondly, from a cosmetic point of view parents prefer their child to have a normally shaped head.[3] In this study we have investigated whether the deformation can be reduced by nursing preterm infants on soft, air filled mattresses.

Subjects and methods

Infants of less than 32 weeks' gestation born at this hospital were included in the study. Within 24 hours of birth they were alternately assigned to be nursed either on a standard, firm, foam filled mattress 12 mm thick supplied with Vickers 79 incubators, or on a soft, air filled mattress. The air mattress was of the type used to detect apnoea (Apnoea alarm Mark 3, Vickers Medical, Hampshire), and was inflated to a pressure of about 2 cm H_2O. Apnoea in infants nursed on the standard mattress was detected by a respiratory sensor taped to the abdomen.

Measurements to determine head shape were made by a single observer (PHTC). The occipitofrontal diameter and biparietal diameter were measured using an anthropometer (Holton Ltd, Dyfed) on days 3, 7, 14, and 21 of life. By not assessing infants during the first three days of life bias due to moulding was minimised. A ratio of the paired measurements of occipitofrontal and biparietal diameter was then calculated as an index of skull flattening and used to assess the progression of the deformity (the higher the ratio, the flatter the shape of the head). Statistical analysis was by unpaired Student's *t* test.

Forty three infants were enrolled into the study. Nine were subsequently excluded, one because of progressive hydrocephalus, and eight because they died before 3 weeks of age. The results of 34 infants were therefore available for analysis. Their gestational ages ranged from 26 to 31 weeks (mean 29·3), and birth weights from 990 to 1850 g (mean 1363). They were equally divided into infants nursed on air mattresses, and standard mattresses, and there were no significant differences in gestational age (means 29·1 and 29·5 weeks) or birth weights (means 1314 and 1412 g). There were also no differences in the mode of delivery, or time spent postnatally in supine and prone positions.

Results

At 3 days of age infants nursed on air mattresses had slightly lower occipitofrontal:biparietal diameter ratios than those cared for on standard mattresses, though the difference was not significant (fig 1). During subsequent weeks the occipitofrontal:biparietal diameter ratio increased in both groups, but the change was most pronounced in those infants nursed on standard mattresses in whom the ratio increased from a mean of 1·37 at three days of age, to 1·48 at 21 days. By contrast, significantly smaller increases

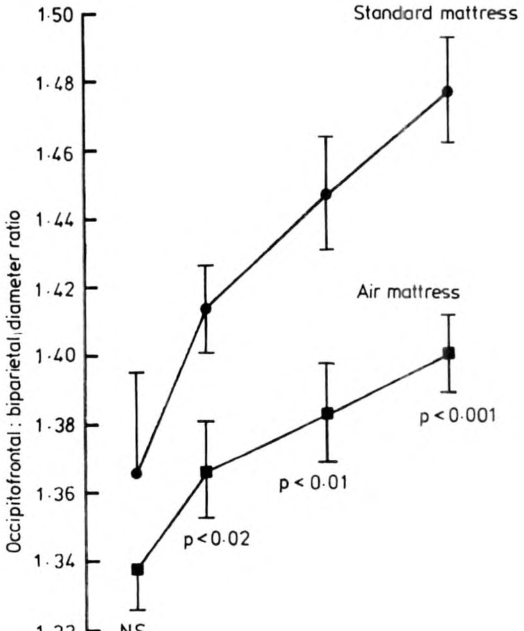

Fig 1 *The change in occipitofrontal:biparietal diameter ratio in preterm infants nursed for the first three weeks of life on either an air mattress or a standard foam mattress (mean and 95% confidence intervals).*

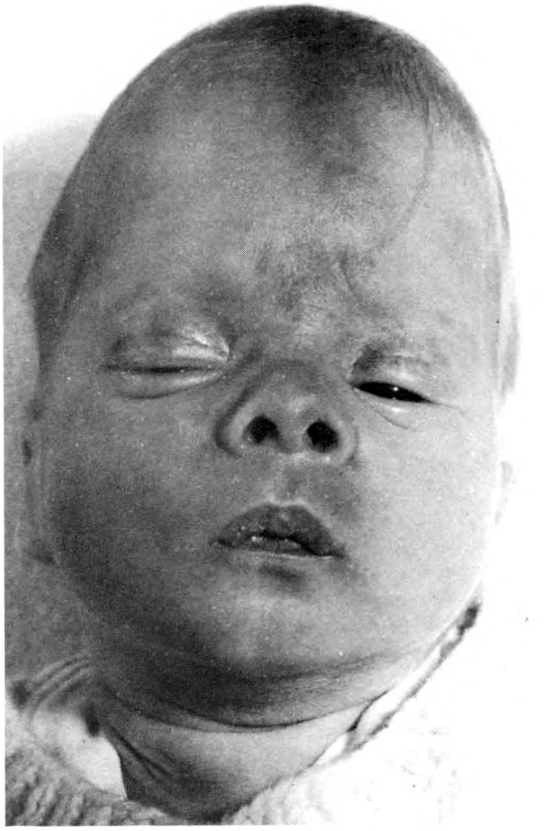

Fig 2 *Effects of postnatal cranial moulding on the appearance of a preterm infant 3 weeks old. Head is flattened (occipitofrontal:biparietal diameter ratio 1·50), forehead high and narrow, and eyes appear laterally placed on long narrow face.*

occurred in infants cared for on air mattresses, from a mean occipitofrontal:biparietal diameter ratio of 1·34 at three days of age to 1·40 at 21 days. The ratio at three weeks of age was thus significantly lower in infants nursed on air mattresses ($p<0{\cdot}001$).

Discussion

The pronounced degree of head flattening that we found in infants nursed on foam mattresses was similar to that reported previously.[1] [2] It was usually accompanied by the other features of postnatal cranial moulding including a high narrow forehead, and eyes which seem to be laterally placed on a long, narrow face (fig 2). This appearance is in stark contrast to the rounded face and head of most babies and is usually thought of as less attractive.[3] We have shown that this deformity can be reduced by nursing preterm infants on soft, air mattresses which should lessen the concern that many parents feel about their infant's dolichocephaly.[4] In addition, the rounder head allows for more accurate interpretation of measurements of head circumference, because they correlate more closely with head growth.[1]

Postnatal cranial moulding can also be reduced by nursing preterm infants on rocking waterbeds.[5] This elaborate equipment is, however, expensive and not generally available. A simpler method was suggested by Marsden, who successfully lessened deformity by using a water pillow in one of a pair of twins.[6] The air mattress, which is commonly used to detect apnoea, is a preferable method of reducing head flattening in preterm infants, as it is simple, cheap, effective, and versatile.

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References

[1] Baum JD, Searls D. Head shape and size of preterm low birthweight infants. *Dev Med Child Neurol* 1971;**13**:576–81.
[2] Largo RH, Duc G. Head growth and changes in head configuration in healthy preterm and term infants during the first six months of life. *Helv Paediatr Acta* 1977;**32**:431–42.
[3] Budreau GK. Postnatal cranial moulding and infant attractiveness: implications for nursing. *Neonatal Network* 1987;**5**:13–19.
[4] Bull MJ, Bryson CQ, Schreiner RL, Lemons JA. Follow up of infants after intensive care. *Perinatology Neonatology* 1986;**9**: 23–8.
[5] Kramer LI, Pierpont ME. Rocking waterbeds and auditory stimuli to enhance growth of preterm infants. *J Pediatr* 1976; **88**:297–9.
[6] Marsden DJ. Reduction of head flattening in preterm infants. *Dev Med Child Neurol* 1980;**22**:507–9.

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Regular review

Prenatal diagnosis and carrier detection in primary immunodeficiency disorders

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The individual forms of inherited immunodeficiency diseases are rare and the true incidence in the United Kingdom is not known. In countries where immunodeficiency registers have been established (Australia, Japan, Spain, and Sweden) an overall incidence of appreciable symptomatic antibody deficiency has been shown to be 1/11 000 live births, and that of severe combined immunodeficiency to be 14/million live births.[1] In the United Kingdom many of the severest forms are not diagnosed until several early deaths have occurred in a family, but with increasing awareness among paediatricians and with better laboratory facilities more of these children will be identified.

Despite rapid progress in the understanding of the underlying pathological mechanism of some of these disorders—for example, chronic granulomatous disease[2] and adenosine deaminase deficiency[3]—treatment is still far from adequate. Children with various forms of severe combined immunodeficiency will die of infections in early childhood unless bone marrow transplantation is undertaken. Bone marrow transplantation from a sibling with identical human leucocyte antigen is an option available only to less than 20% of cases, and mismatched HLA bone marrow transplantation is still associated with high morbidity and mortality.[4] Patients with Wiskott-Aldrich syndrome and ataxia telangiectasia may survive into adulthood with appropriate support, but they have increasing morbidity and a much higher risk of developing lymphoreticular malignancy.[5] With early diagnosis and aggressive treatment patients with chronic granulomatous disease are surviving into adulthood with varying degrees of chronic organ dysfunction, but many still die young of fungal infections, especially those caused by aspergillus.[6-7] For patients with X-linked agammaglobulinaemia, immunoglobulin replacement treatment has prevented many of the infectious complications, but affected patients still develop chronic lung damage and may still be prone to fatal echoviral infection.[8]

In families who have already had a severely affected child, therefore, genetic counselling with the possibility of carrier detection and prenatal diagnosis is an indispensable part of good management. The severe inherited immunodeficiency disorders are listed in table 1 under either X-linked or autosomal recessive inheritance. With the recent unravelling of the underlying cellular, biochemical, or genetic defects of such disorders, prenatal diagnosis and carrier detection are becoming possible in many different ways.

Methods of prenatal diagnosis and carrier detection

GENE TRACKING

Direct analysis of the deoxyribonucleic acid (DNA) by Southern blotting[9] has enormous potential for genetic prediction in diseases of strict Mendelian

Table 1 *Severe inherited immunodeficiency disorders*

Autosomal recessive inheritance

- Severe combined immunodeficiency due to:
  - Adenosine deaminase deficiency
  - Purine nucleoside phosphorylase deficiency
  - Absence of HLA class I or II, or both (bare lymphocyte syndrome)
  - Reticular dysgenesis
  - Severe combined immunodeficiency in association with short limbed dwarfism

- Leucocyte adhesion deficiency (leucocyte function antigen-1 deficiency)
- Ataxia telangiectasia
- Chronic granulomatous disease
- Chédiak-Higashi syndrome

X-linked inheritance

- X-linked agammaglobulinaemia
- Severe combined immunodeficiency
- Wiskott-Aldrich syndrome
- Chronic granulomatous disease
- X-linked hyperimmunoglobulinaemia M
- X-linked lymphoproliferative disease
- X-linked properdin deficiency
- X-linked agammaglobulinaemia with growth hormone deficiency

inheritance. This permits prenatal diagnosis during the first trimester by analysis of chorionic villus biopsy specimens. The method used is called gene tracking.[10] In most diseases, allelic genetic heterogeneity precludes elucidation of the mutation in all families, and gene tracking asks the question: 'Has the fetus or relative inherited the same relevant chromosomal region as a previously affected family member?'.[10] Gene tracking requires a gene specific or closely linked DNA probe that detects a restriction fragment length polymorphism, which is used to distinguish each chromosome of the pair in key family members. It is independent of allelic genetic heterogeneity but only useful in families whose key members are heterozygous for that restriction fragment length polymorphism.

This approach is especially useful in diseases in which the underlying defect is unknown. As soon as a close linkage is established between the disease and a DNA probe, gene tracking may potentially be used in prenatal diagnosis and detection of carriers without knowing the defect involved. Recently, genetic linkage with restriction fragment length polymorphisms has been established for X-linked agammaglobulinaemia,[11–13] X-linked severe combined immunodeficiency,[14] X-linked hyperimmunoglobulinaemia M,[15] Wiskott-Aldrich syndrome,[16] and X-linked lymphoproliferative disease[17] (table 2).

For X-linked severe combined immunodeficiency (a disease characterised by a block in early T cell differentiation) the gene has been mapped to Xq11-q13 by linkage analysis with restriction fragment length polymorphisms in nine families.[14] Similarly, the gene for the Wiskott-Aldrich syndrome was mapped to the pericentric region of the X chromosome in 10 families.[16] There is no evidence of non-allelic genetic heterogeneity in these two diseases so far,[14 16] and the linked probes established in these studies (cpX73 (DXS159) for X-linked severe combined immunodeficiency and L1·28 (DXS7) for the Wiskott-Aldrich syndrome) may be used for prenatal diagnosis and detection of carriers with the proviso that not all families are informative for the known probes. Furthermore, sporadic cases or pedigrees without key members for analysis may also present difficulties with this approach. In the past it has not been possible to detect carriers in these two disorders, though prenatal diagnosis has been possible for severe combined immunodeficiency by analysis of the lymphocyte subpopulation of fetal blood in mid-trimester.[18]

For X-linked agammaglobulinaemia (a disease characterised by a block in differentiation from pre-B cell to B cell development[19]) there are three studies mapping the gene to Xq21·3–22.[11–13] There seems, however, to be a degree of non-allelic genetic heterogeneity[12 20] that has complicated the approach of gene tracking in prenatal diagnosis and carrier detection. Fortunately on the present evidence the proportion of unlinked families to Xq21·3–22 seems to be about 10%.[21] A method has been developed for the calculation of genetic risk that takes non-allelic genetic heterogeneity into account.[21] With this approach it is still possible to use the linked probes S21 (DXS17) and pXG12 (DXS94) for genetic counselling,[13] and it has been used successfully to exclude the diagnosis of X-linked agammaglobulinaemia in a male fetus in the first trimester.[21]

For X-linked lymphoproliferative syndrome and X-linked hyperimmunoglobulinaemia M the linkage was established in only one family with each disease. The gene for X-linked lymphoproliferative syndrome was mapped to Xq24–27 and that for X-linked hyperimmunoglobulinaemia to Xq24–27 as well.[15 17] More information will be needed in further families to establish whether there is non-allelic genetic heterogeneity, and until more is known the use of gene tracking for genetic counselling is probably not recommended in these two disorders.

For X-linked chronic granulomatous disease, the

Table 2 *Severe inherited immunodeficiency disorders in which gene tracking may potentially be used in prenatal diagnosis and detection of carriers*

Disease	*Genetic localisation*	*Probes linked to disease*
X-linked agammaglobulinaemia	Xq21·3–q22	S21 (DXS17) pXG12 (DXS94)
X-linked severe combined immunodeficiency	Xq11–q13	cpX73 (DXS159)
Wiskott-Aldrich syndrome	Xp11–p11·3	L1·28 (DXS7)
X-linked hyperimmunoglobulinaemia M	Xq24–q27	43–15 (DXS42)
X-linked lymphoproliferative syndrome	Xq24–q27	43–15 (DXS42)
X-linked chronic granulomatous disease	Xp21·1	Gene specific
Properdin deficiency	Xp21·1–cen	L1·28 (DXS7)
X-linked agammaglobulinaemia with growth hormone deficiency	Unknown	Unknown

gene was mapped to Xp21 (22), cloned,[23] and identified as the β chain of cytochrome b 245 [24–25] but no restriction fragment length polymorphism has been identified with the gene specific probe yet to make gene tracking possible. Only about 10% of the mutations of X-linked chronic granulomatous disease are caused by a gene deletion[23] that can be detected by the gene specific probe; hence only in such families would it be possible to use the gene specific probe for prenatal diagnosis in the first trimester.

MEASUREMENT OF GENE PRODUCT

There are four severe inherited immunodeficiency disorders, all of autosomal recessive inheritance, in which the underlying biochemical or cellular defects are known and the normal gene products can be measured. These are adenosine deaminase deficiency, purine nucleoside phosphorylase deficiency, bare lymphocyte syndrome, and leucocyte adhesion deficiency (leucocyte function antigen-1 deficiency). Adenosine deaminase deficiency results in severe combined immunodeficiency and accounts for about 25% of the autosomal recessive forms,[26] and purine nucleoside phosphorylase deficiency results in a predominantly T cell defect.[27] Both enzymes play a part in the purine degradation pathway and their activities in either fetal red cells or amniotic fluid cells can be assayed rapidly to permit prenatal diagnosis in the mid-trimester as their activities at this stage of gestation are the same as those found in children and adults.[28] In an affected fetus there will be little or no enzymatic activity with accumulation of the deoxynucleotides in the red cells, and immunological investigation of fetal blood would show profound lymphopenia, severely depleted T cells, and no phytohaemagglutinin induced proliferation.[28, 29]

Recently it became possible to measure adenosine deaminase and purine nucleoside phosphorylase in chorionic villi, so permitting prenatal diagnosis of such deficiencies in the first trimester.[29 30] There is still a problem, however, with this approach in some cases in distinguishing between the homozygous affected state and heterozygous normal[30] because 10–15% of infants with severe combined immunodeficiency who are deficient in adenosine deaminase still have low but detectable adenosine deaminase activity in lymphoid cells.[31] The most reliable prenatal diagnosis of adenosine deaminase deficiency is therefore still by analysis in mid-trimester of fetal blood in which enzyme activities, deoxynucleotides, T cell subpopulations, and proliferative responses may be measured.[28] Detection of carriers is also possible by finding intermediate activity of the enzyme in the subject concerned.

In the bare lymphocyte syndrome the deficient gene products are the HLA molecules—either class I or II—which were shown to be secondary to defective synthesis[32] associated with an abnormal transactive regulatory gene outside the major histocompatibility complex.[33] This syndrome results in a combined immunodeficiency with repeated bacterial and viral infections. Two affected fetuses have been diagnosed in mid-trimester by membrane immunofluorescence on blood lymphocytes and monocytes using specific monoclonal antibodies for HLA class I and II molecules.[34] Leucocyte adhesion deficiency presents as delayed cord separation and fulminating bacterial infection. The basic genetic defect is the result of the absence of synthesis of the mature β chain subunit of the leucocyte function antigen-1 heterodimer.[35] As the β chain is essential for assembly of the α/β molecule on the cell surface, it should be possible to perform prenatal diagnosis in the mid-trimester using monoclonal antibodies for leucocyte function antigen-1 α and β chains. This has not yet been attempted. It is not possible to detect carriers of either bare lymphocyte syndrome or leucocyte function antigen-1 deficiency as they express the HLA and leucocyte function antigen-1 molecules normally.

CELL FUNCTION OR DYSFUNCTION

The knowledge that the superoxide generating system in granulocytes is already functionally complete by 16 weeks' gestation enables one to diagnose X-linked chronic granulomatous disease prenatally in mid-trimester using the nitroblue tetrazolium test, which measures superoxide production.[36–37] Normal fetal granulocytes function as well as normal adult granulocytes, and there are clear cut separations in these functions among an affected, a heterozygous, and a normal subject.[36] This is still the most reliable method of prenatal diagnosis of this disease except in cases where gene deletion can be shown by the gene specific probe. Even when a restriction fragment length polymorphism is found in the future, there will still be families in which gene tracking is not possible because the key members are not heterozygous for that restriction fragment length polymorphism and the simple functional nitroblue tetrazolium assay for prenatal diagnosis will still be useful.

X-linked lymphoproliferative syndrome is characterised by increased susceptibility to fatal Epstein-Barr virus infection, acquired agammaglobulinaemia, and lymphoma.[38] Asymptomatic female carriers of X-linked lymphoproliferative syndrome have abnormal antibody responses to Epstein-Barr virus[39] and serial monitoring of antibody titres over a year or more may be of predictive use in evaluating female subjects at risk of being carriers.

Another condition, ataxia telangiectasia, has been diagnosed prenatally by showing cellular dysfunction.[40] The diagnosis of an affected fetus was based on increased spontaneous chromosomal breakage in the amniotic cells and the presence of an abnormal clone with a 14/5 translocation. Prenatal exclusion has also been performed by analysis of chromosomal breakage of lymphocytes after small doses of irradiation.[41]

PRENATAL DIAGNOSIS BY SHOWING ABNORMALITIES OF A CELL SERIES

Having defined the ontogeny of various lymphocyte subpopulations in fetal life (table 3) it is possible to diagnose immunodeficiency disorders with absence of certain lymphocyte series prenatally.[28 42–43] The technique depends on the ability to analyse small amounts of fetal blood obtained in mid-trimester, and was initially perfected for analysing mononuclear cell populations stained with fluorescent antibody on a fluorescent activated cell sorter.[42] More recently this had been adapted to allow enumeration on slides after peroxidase staining (Linch and Levinsky, unpublished observations). It is primarily useful in the prenatal diagnosis of severe combined immunodeficiency syndrome irrespective of its cause, as long as the abnormality in the lymphocyte cell series has been well defined in an affected child in the family seeking prenatal diagnosis.[28 42–43]

It is not advisable to use this technique for the prenatal diagnosis of X-linked agammaglobulinaemia because of the variability in the numbers of B cells in a normal fetus in mid-trimester.[42] If normal numbers of B cells were seen, however, this would be sufficient to exclude the diagnosis.[43] In the Chédiak-Higashi syndrome the neutrophils have large inclusion bodies in the cytoplasm[44] and as enough neutrophils may be found in fetal blood at 18–20 weeks' gestation, prenatal diagnosis of this autosomal recessive condition should be possible and it has already been excluded in three fetuses.[43]

CARRIER DETECTION BY EXPLOITING THE EFFECTS OF LYONISATION

The Lyon hypothesis states that permanent inactivation of one of the two X chromosomes occurs at random in every somatic cell in the female subject early in embryogenesis.[45] The pattern of inactivation of X chromosomes is then transmitted in a stable fashion to all progency cells. Therefore in a female carrier of an X-linked immunodeficiency disorder there will be two distinct populations of mature cells if the gene is not essential for development of that cell type, but there will only be one population of mature cells with the active normal X-chromosome that confers selective advantage if the gene is essential for development of that cell type.

In X-linked chronic granulomatous disease the gene is not essential for the development of the neutrophils, but pivotal in enabling effective killing of bacteria. There are therefore two populations of neutrophils in a female carrier, one that can reduce nitroblue tetrazolium and one that cannot.[36] This is the basis of carrier detection of X-linked chronic granulomatous disease by the nitroblue tetrazolium test. The female carriers are prone to a lupus like syndrome,[46] and severe lyonisation can lead to a clinical picture of chronic granulomatous disease in some cases.[47]

In X-linked agammaglobulinaemia the gene is essential for the development of B cells. There is therefore only one population of mature B cells with the active normal X chromosome in a female carrier.[48 49] This was first established by finding mature B cells in female carriers of X-linked agammaglobulinaemia who were heterozygous for

Table 3 *Lymphocyte ontogeny in humans*

T cells	*No of weeks after conception*	*B cells*
Epithelial thymus	5	
	6	
	7	$C\mu^+$ pre-B cells in liver
Thymus becomes lymphoid	8	
	9	$S\mu^+$ B cells in liver
Corticomedullary differentiation in thymus	10	$S\gamma^+$ B cells in liver
	11	$S\alpha^+$ B cells in liver
Hassall's corpuscles appear	12	Most μ^+ B cells have δ
	13	
T cells in spleen	14	B cells in blood and spleen
T cells in blood	15	

glucose-6-phosphate dehydrogenase and who only expressed one of its isoenzymes, though their T cells and granulocytes expressed both isoenzymes.[48] Using recombinant DNA probes, which simultaneously detect restriction fragment length polymorphisms and patterns of methylation of X-chromosome genes, Fearon *et al*[49] showed the pattern of random X-inactivation in T cells and granulocytes in female carriers of X-linked agammaglobulinaemia, but it did not occur in B cells where only one of the two X chromosomes was preferentially active. Using the same technique to examine X-chromosome inactivation patterns female carriers of X-linked severe combined immunodeficiency and of the Wiskott-Aldrich syndrome can be detected by examining their T cells, which show patterns of non-random X-inactivation.[50–51] This strongly supports the hypothesis that T cells have the intrinsic defect in both disorders, in which the genes are essential for the development of T cells. This technique is, however, limited to about 50% of the female subjects who are heterozygous for the DNA probes used, which are from the 5′ end of the genes encoding the phosphoglycerate kinase and hypoxanthine phosphoribosyltransferase.

Conclusion

The severe immunodeficiency disorders listed in table 1 are all 'single gene' disorders that are rare, and therefore prenatal diagnosis and detection of carriers will be limited to families which have already had an affected child. When further progress is made in elucidating the basic biochemical defect in these disorders, precise prenatal diagnosis, or carrier detection, or both, will become possible by measurement of the absent or aberrant protein or enzymes. Before that is possible, however, the strategy for diseases in which the underlying defect is unknown remains mapping of the gene by linkage study using restriction fragment length polymorphism; this would permit prenatal diagnosis and detection of carriers. There are, however, constraints associated with analysis of restriction fragment length polymorphism and there is always less than 100% certainty if gene specific probes are not used.

The purpose of the detection of carriers should be to assist informed decision making before pregnancy. Accurate information about possible risks will permit some couples to avoid pregnancies that are at high risk, while other couples may decide to embark on pregnancies that they would, without this knowledge, have avoided.[52]

The purpose of prenatal diagnosis is to provide the option of not continuing with an abnormal pregnancy, but should the mother wish to continue with the pregnancy the prenatal diagnosis allows the family time to prepare to care for an affected child[52] or to attempt appropriate treatment as soon as possible after birth. This is particularly relevant for those with severe combined immunodeficiency in which both matched and mismatched bone marrow transplants are curative, and the results are successful in about 90% of HLA matched, and 50–60% of HLA haploidentical, transplants.[4]

Although an increasing number of these conditions are amenable to diagnosis in the first trimester by analysis of chorionic villus biopsy specimens, at present most can only be diagnosed by analysing fetal blood taken at 18–20 weeks' gestation. Legislation to limit the time of abortion to the eighteenth week would deny such families the option of prenatal diagnosis.

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References

[1] Hosking CS, Roberton DM. Epidemiology and treatment of hypogammaglobulinemia. *Births Defects* 1983;**19**:223–7.

[2] Segal AW, Cross AR, Garcia RC, *et al.* Absence of cytochrome b 245 in chronic granulomatous disease. *N Engl J Med* 1983;**308**:245–51.

[3] Morgan G, Levinsky RJ, Hugh-Jones K, Fairbanks LD, Morris GS, Simmonds HA. Heterogeneity of biochemical, clinical and immunological parameters in severe combined immunodeficiency due to adenosine deaminase deficiency. *Clin Exp Immunol* 1987;**70**:491–9.

[4] Fischer A, Griscelli C, Friedrich W, *et al.* Bone-marrow transplantation for immunodeficiencies and osteopetrosis: European survey, 1968–1985. *Lancet* 1986;ii:1080–4.

[5] Spector BD, Perry CS III, Kersey JH. Genetically determined immunodeficiency diseases (GDID) and malignancy: report from the immunodeficiency-cancer registry. *Clin Immunol Immunopathol* 1979;**11**:21–9.

[6] Cohen MS, Isturiz RE, Malech HL, *et al.* Fungal infection in chronic granulomatous disease. The importance of the phagocyte in defense against fungi. *Am J Med* 1981;**71**:59–66.

[7] Koch C, Valerius NH, Pedersen FK, Heilmann C, Faber V. Fungal infections in chronic granulomatous disease. In: Vossen J, Griscelli C, eds. *Progress in immunodeficiency research and therapy.* Vol II. Amsterdam: Elsevier, 1986;147–50.

[8] Lederman HM, Winkelstein JA. X-linked agammaglobulinemia: an analysis of 96 patients. *Medicine* 1985;**64**:145–56.

[9] Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* 1975;**98**:503–17.

[10] Pembrey ME. Applications and limitations of direct DNA analysis in genetic prediction. *J Inherited Metab Dis* 1986;**9** (suppl 1):38–48.

[11] Kwan SP, Kunkel L, Bruns GP, Wedgwood RJ, Latt S, Rosen FS. Mapping of the X-linked agammaglobulinemia locus by use

of restriction fragment-length polymorphism. *J Clin Invest* 1986;**77**:649–52.

[12] Mensink EJBM, Thompson A, Schot JDL, van de Greef WMM, Sandkuyl LA, Schuurman RKB. Mapping of a gene for X-linked agammaglobulinemia and evidence for genetic heterogeneity. *Hum Genet* 1986;**73**:327–32.

[13] Malcolm S, de Saint Basile G, Arveiler B, *et al.* Close linkage of random DNA fragments from Xq21·3–22 to X-linked agammaglobulinaemia (XLA). *Hum Genet* 1987;**77**:172–4.

[14] de Saint Basile G, Arveiler B, Oberle I, *et al.* Close linkage of the locus for X chromosome-linked severe combined immunodeficiency to polymorphic DNA markers in Xq11–q13. *Proc Natl Acad Sci USA* 1987;**84**:7576–9.

[15] Mensink EJBM, Thompson A, Sandkuyl LA, *et al.* X-linked immunodeficiency with hyperimmunoglobulinemia M appears to be linked to the DXS 42 restriction fragment length polymorphism locus. *Hum Genet* 1987;**76**:96–9.

[16] Peacocke M, Siminovitch KA. Linkage of the Wiskott-Aldrich syndrome with polymorphic DNA sequences from the human X chromosome. *Proc Natl Acad Sci USA* 1987;**84**:3430–3.

[17] Skare JC, Milunsky A, Byron KS, Sullivan JL. Mapping the X-linked lymphoproliferative syndrome. *Proc Natl Acad Sci USA* 1987;**84**:2015–8.

[18] Linch DC, Levinsky RJ. Prenatal diagnosis of immunodeficiency disorders. *Br Med Bull* 1983;**39**:399–404.

[19] Pearl E, Vogler LB, Okas AJ, Crist WM, Lawton III AR, Cooper MD. B lymphocyte precursors in human bone marrow: an analysis of normal individuals and patients with antibody-deficiency states. *J Immunol* 1978;**120**:1169–75.

[20] Ott J, Mensink EJBM, Thompson A, Schot JDL, Schuurman RKB. Heterogeneity in the map distance between X-linked agammaglobulinemia and a map of nine RFLP loci. *Hum Genet* 1986;**74**:280–3.

[21] Lau YL, Levinsky RJ, Malcolm S, Goodship JA, Winter R, Pembrey ME. Genetic prediction in X-linked agammaglobulinaemia. *Am J Med Genet* 1988 (in press).

[22] Baehner RL, Kunkel LM, Monaco AP, *et al.* DNA linkage analysis of X chromosome-linked chronic granulomatous disease. *Proc Natl Acad Sci USA* 1986;**83**:3398–401.

[23] Royer-Pokora B, Kunkel LM, Monaco AP, *et al.* Cloning the gene for an inherited human disorder—chronic granulomatous disease—on the basis of its chromosomal location. *Nature* 1986;**322**:32–8.

[24] Dinauer MC, Orkin SH, Brown R, Jesaitis AJ, Parkos CA. The glycoprotein encoded by the X-linked chronic granulomatous disease locus is a component of the neutrophil cytochrome b complex. *Nature* 1987;**327**:717–20.

[25] Teahan C, Rowe P, Parker P, Totty N, Segal AW. The X-linked chronic granulomatous disease gene codes for the beta-chain of cytochrome b_{245}. *Nature* 1987;**327**:720–1.

[26] Gelfand EW, Dosch H-H. Diagnosis and classification of severe combined immune deficiency disease. In: Wedgewood RJ, Rosen FS, Paul NW, eds. *Primary immunodeficiency diseases.* New York: Alan R Liss, 1983:65–72.

[27] Carapella De Luca E, Stegagno M, Dionisi Vici C, *et al.* Prenatal exclusion of purine nucleoside phosphorylase deficiency. *Eur J Pediatr* 1986;**145**:51–3.

[28] Linch DC, Levinsky RJ, Rodeck CH, Maclennan KA, Simmonds HA. Prenatal diagnosis of three cases of severe combined immunodeficiency: severe T cell deficiency during the first half of gestation in fetuses with adenosine deaminase deficiency. *Clin Exp Immunol* 1984;**56**:223–32.

[29] Perignon JL, Durandy A, Peter MO, Freycon F, Dumez Y, Griscelli C. Early prenatal diagnosis of inherited severe immunodeficiencies linked to enzyme deficiencies. *J Pediatr* 1987;**111**:595–8.

[30] Dooley T, Fairbanks LD, Simmonds HA, *et al.* First trimester diagnosis of adenosine deaminase deficiency. *Pregnat Diagn* 1987;**7**:561–65.

[31] Simmonds HA, Fairbanks LD, Morris GS, Webster DR, Morgan G, Levinsky RJ. Correlation between purine levels, clinical and immunological status in ADA deficiency. In: Nyhan WL, Thompson LF, Watts RWE, eds. *Purine and pyrimidine in man.* Plenum, New York: Plenum 1986:93–101.

[32] Lisowska-Grospierre B, Charron DJ, de Preval C, Durandy A, Griscelli C, Mach B. A defect in the regulation of MHC class II gene expression in HLA-DR negative lymphocytes from patients with combined immunodeficiency syndrome. *J Clin Invest* 1985;**76**:381–5.

[33] de Preval C, Lisowska-Grospierre B, Loche M, Griscelli C, Mach P. The lack of expression of HLA class II antigens in severe immunodeficiency reveals the existence of a transacting class II regulatory gene, unlinked to the MHC. *Nature* 1985;**318**:291–3.

[34] Durandy A, Cerf-Bensussan N, Dumez Y, Griscelli C. Prenatal diagnosis of severe combined immunodeficiency with defective synthesis of HLA molecules. *Prenat Diagn* 1987;**7**:27–34.

[35] Lisowska-Grospierre B, Bohler M-C, Fischer A, Mawas C, Springer TA, Griscelli C. Defective membrane expression of the LFA-1 complex may be secondary to the absence of the β chain in a child with recurrent bacterial infection. *Eur J Immunol* 1986;**16**:205–8.

[36] Newburger PE, Cohen HJ, Rothchild SB, Hobbins JC, Malawista SE, Mahoney MJ. Prenatal diagnosis of chronic granulomatous disease. *N Engl J Med* 1979;**300**:178–81.

[37] Levinsky RJ, Harvey BAM, Rodeck CH, Soothill JF. Phorbol myristate acetate stimulated NBT test: a simple method suitable for antenatal diagnosis of chronic granulomatous disease. *Clin Exp Immunol* 1983;**54**:595–8.

[38] Hamilton JK, Paquin LA, Sullivan JL, *et al.* X-linked lymphoproliferative syndrome registry report. *J Pediatr* 1980;**96**: 669–73.

[39] Sakamoto K, Seeley K, Lindsten T, *et al.* Abnormal anti-Epstein-Barr virus antibodies in carriers of the X-linked lymphoproliferative syndrome and in females at risk. *J Immunol* 1982;**128**:904–7.

[40] Shaham M, Voss R, Becker Y, Yarkoni S, Ornoy A, Kohn G. Prenatal diagnosis of ataxia telangiectasia. *J Pediatr* 1982;**100**:134–7.

[41] Giannelli F, Avery JA, Pembrey ME, Blunt S. Prenatal exclusion of ataxia-telangiectasia. In: Bridges BA, Harnden DG, eds. *Ataxia-telangiectasia—a cellular and molecular link between cancer, neuropathy and immune deficiency.* London: John Wiley and Sons, 1982:393–400.

[42] Linch DC, Beverley PCL, Levinsky RJ, Rodeck CH. Phenotypic analysis of fetal blood leucocytes: potential for prenatal diagnosis of immunodeficiency disorders. *Prenat Diagn* 1982;**2**:211–8.

[43] Durandy A, Dumez Y, Griscelli C. Prenatal diagnosis of severe inherited immunodeficiencies: a five year experience. In: Progress in immunodeficiency research and therapy II. Vossen J, Griscelli C, eds. Amsterdam: Elsevier, 1986:323–7.

[44] White JG. Chediak-Higashi syndrome. Cytoplasmic sequestration in circulating leucocytes. *Blood* 1967;**29**:435–51.

[45] Lyon MF. Gene action in the X-chromosome of the mouse (Mus musculus L). *Nature* 1961;**190**:372–3.

[46] Brandrup F, Koch C, Petri M, Schiodt M, Johansen KS. Discoid lupus erythematosus-like lesions and stomatitis in female carriers of X-linked chronic granulomatous disease. *Br J Dermatol* 1981;**104**:495–505.

[47] Mills EL, Rholl KS, Quie PG. X-linked inheritance in females with chronic granulomatous disease. *J Clin Invest* 1980;**66**: 332–40.

[48] Conley ME, Brown P, Pickard AR, *et al.* Expression of the gene defect in X-linked agammaglobulinaemia. *N Engl J Med* 1986;**315**:564–7.

[49] Fearon ER, Winkelstein JA, Civin CI, Pardoll DM, Vogelstein B. Carrier detection in X-linked agammaglobulinaemia by analysis of X-chromosome inactivation. *N Engl J Med* 1987;**316**:427–31.

[50] Goodship J, Malcolm S, Lau YL, Pembrey ME, Levinsky RJ. Use of X chromosome inactivation analysis to establish carrier status for X-linked severe combined immunodeficiency. *Lancet* 1988;**1**:729–32.

[51] Kohn DB, Fearon ER, Winkelstein JA, Vogelstein B, Blaese RM. Wiskott-Aldrich syndrome (WAS) carrier detection by X-chromosome inactivation analysis. *Pediatr Res* 1987;**21**:313A.

[52] King's Fund Forum consensus statement: screening for fetal and genetic abnormality. *Br Med J* 1987;**295**:1551–3.

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ook reviews

inatal medicine: Problems and Con- versies. Edited by G C Di Renzo and F Hawkins. Pp 237: $56·50 hardback. blished by Cortina Verona but distri- ted worldwide (except Italy) by Raven ss, 1986. ISBN 88–85037–47–X.

rinatal Medicine: Problems and Con- versies is a collection of short mono- phs collected by Gian Carlo Di Renzo d Denis F Hawkins, and they are dedi- ed to the late Professor Montanari who ld several prestigious chairs in obstetrics d gynaecology in Italy.

The editors have collected 35 articles and ganised them in four sections: monitor- of pregnancy, assessment of fetal lung turity, preterm labour, and psycho- matic factors in perinatal medicine. All authors have an international reputation. turally the 'home team' is strongly re- esented, just under half being Italian. e other authors range over Europe and rth America and are leaders in their lds.

I particularly like the brevity of the icles, the longest being 10 pages on fetal temic function and behaviour (by J W ladimiroff), which described the use of rasound to measure cardiac function, nal function, fetal breathing, and move- nt. Most articles were five to seven ges long and all had a short list of key ferences (10–15). The article on ante- tal cardiotachography, fetal sonic stimu- ion, fetal electro-encephalography, fetal transcutaneous oxygen tension, and subcutaneous oxygen electrode were all liberally illustrated with traces. In fact there is a wealth of wiggly lines.

As a neonatal paediatrician I found this a fascinating collection of monographs covering many of the esoteric aspects of fetal medicine and pregnancy. In one volume one has the oxytocin stress test re-evaluated (by E Ferrazzi, P Bozzetti, and G Pardi); Charles Rodeck looking at the fetus; and G Vecchietti and F Borruto telling us how to medically and surgically treat fetuses antenatally. There is also D V Walters explaining why the fetus and newborn baby do not drown in their own lung fluid; T K A B Eskes giving some scientific evidence that home delivery improves the cord blood gases; and R C Goodlin giving us his views on 'sex' in pregnancy.

I have two criticisms: the title should be prenatal and not perinatal, and secondly, the most recent reference quoted was 1983. This excellent multiauthor collection of monographs is now distinctly 'postmature'.

G M DURBIN

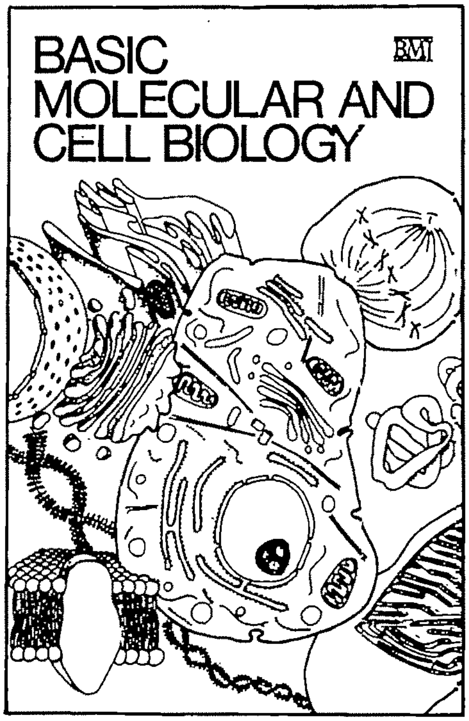
The Inherited Metabolic Diseases. By J B Holton. Pp 462: £50 hardback. Churchill Livingstone, 1987. ISBN 0-443-03195-9.

The editor has attempted an ambitious project with this volume. His aim is to provide a comprehensive account of inborn errors of metabolism suitable to the needs of a wide range of readers, from the student or clinician in training on one hand to the research scientist on the other. In many ways the book is a success. It is well produced and presented. Unlike many multiauthor works it is very easy to read and its figures and diagrams are well chosen and clear. The authors of the individual chapters are recognised within the field and cover their subjects to a high standard.

While the content of the book is therefore satisfactory, unfortunately the subject is one in which rapid advances are being made and already some of the chapters are out of date. This is most noticeable when one reads the reference lists at the end of each chapter, for many the most recent references are already some three years old. Perhaps an addendum at the proof stage could have prevented this. This may not detract from the value of the book to the student or clinician in training, but will certainly limit its value to the research worker. In addition to this the peroxisomal disorders are hardly considered and I am sure that if the book were at the planning stage now a chapter on the inborn errors of this organelle would have been included. While hospital libraries will buy this volume the price of £50 may unfortunately deter the individual from purchase.

J E WRAITH

One revolution leads to another . . .



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Classification of perinatal death

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SUMMARY Three paediatric pathologists, one perinatal paediatrician, one obstetrician, and one epidemiologist separately used information collected on 239 babies in an attempt to validate the Wigglesworth classification of perinatal deaths. This was first done using clinical data only, then using the combination of clinical and gross necropsy findings and finally using clinical, gross necropsy, histological and any other information (for example, chromosome analyses, microbiological investigations). Only 14 (6%) of deaths changed groups within the Wigglesworth classification when gross necropsy findings were considered as well as clinical findings, and altogether only 21 (9%) changed classification when complete investigations were available. There was an unacceptable amount (15%) of disagreement between the classifiers, largely the result of failure to comply with the rules laid down for classification. We set out amendments to Wigglesworth's original definitions to clarify certain ambiguities.

The number of classification systems of the causes of perinatal death have increased over the years. Obstetricians[1 2] have derived classifications that differ substantially from those of pathologists.[3–7] Paediatricians tend either to quote basic statistics,[8] or to use classifications derived by pathologists rather than obstetricians.[6 7] Despite the multiplicity of classifications, and firm but divergent opinions about which may be best, there has been little assessment of the repeatability or the usefulness of any of them.

The aim of any classification must be to derive strategies to understand the reasons for, and ultimately prevent, perinatal mortality. Wigglesworth argued that this was the only valid reason for a classification.[5] He suggested that any classification should be as simple as possible and presented in such a way that the results give clear indications of priorities for prevention, and that the classification should be used to indicate the areas of health care provision most in need of alteration. He put forward a simple classification in which he allotted deaths to one of only five categories. It was devised so that it could be applied with reservation to cases where necropsy investigation had not been undertaken, although he acknowledged that necropsy would permit the most accurate categorisation.

The simplicity of the classification is most attractive and the fact that it can be used when necropsy has not been undertaken is helpful. Nevertheless, problems were encountered when it was used in one specialised unit[9] and other problems were encountered by one of us (JWK) in attempting to apply it to a regional study of perinatal mortality with a high necropsy rate.

The present study was set up with three objectives: firstly, to define more accurately the groups of the classification, particularly in respect of problems already encountered; secondly, to investigate the extent to which classification of cases was changed by necropsy; and thirdly, to assess the reproducibility of the classification when undertaken by people with an interest in perinatal medicine and pathology but with different professional backgrounds.

Method

The six authors (three perinatal pathologists, one perinatal paediatrician, one obstetrician, and one epidemiologist) met first to discuss problems already identified in the Wigglesworth classification and to define its groups more clearly. At a subsequent meeting each member of the group was given the clinical, gross necropsy findings, and details of further investigations of 233 perinatal deaths that had been collected as part of a multicentre study of perinatal deaths. Each member of the group was asked to use the Wigglesworth classification and classify the death firstly on the clinical information alone, secondly on the clinical information together with the findings from the macroscopic examination at necropsy (which included the dissection of organs), and thirdly to classify the death taking account not only of the clinical and necropsy findings but also the histological and microbiological examinations, and chromosome analyses.

Each member of the team carried out the classification without knowledge of the classification made by the other members. After 89 perinatal deaths had been classified, the team met to discuss problems encountered and to compare results of the initial classification exercise. At this stage there was substantial disagreement. For each case over which there was disagreement, extensive discussion took place to try and define more clearly the rules of the classification. Once these rules had been delineated more clearly, the team was presented with a further 144 cases to classify. Before this, the obstetrician (IM) had classified the 233 deaths using the Aberdeen classification.[2]

The following analyses of the data were carried out: (i) identification of cases where there was still disagreement concerning the appropriate Wigglesworth grouping; (ii) a study of all cases where necropsy and other results had made a difference to the classification; (iii) a comparison of the classifications made by all six observers of the last 144 cases (to give an estimate of interobserver reliability); and (iv) a comparison of the Aberdeen classification with the Wigglesworth classification, after taking into account all necropsy and other findings.

Results

CLARIFICATION OF THE CLASSIFICATION

Group 1

The original Wigglesworth group 1 was described as 'normally formed macerated stillbirth'. After discussion the group was renamed 'deaths before the start of labour'. In the absence of other evidence, the presence of maceration should be taken to indicate that death preceded the onset of labour. Antepartum fetal deaths with lethal malformations were excluded, as were the miscellaneous disorders (see group 5).

Much discussion concerned the distinction between lethal and potentionally lethal malformations and minor malformations. It was suggested that macerated infants with minor isolated lesions (such as a small ventricular septal defect) should remain in this group, but that any infants with multiple minor anomalies (for example, cleft lip and a skeletal defect) should be classified in group 2. Because many antepartum fetal deaths are associated with abruption, it was thought that a subgroup might be appropriate.

Group 2

Group 2 was originally described as 'congenital malformations (stillbirth or neonatal death)'. Infants with minor or potentially treatable minor malformations should not be included here unless they formed part of a complex of at least two malformations and they had died before the start of labour. Deformations—that is abnormalities of form secondary to a fetal disease or functional impairment—could also be included here. Thus pulmonary hypoplasia that follows oligohydramnios may be included, although we thought that this difficult diagnosis should always be supported by the presence of other deformations or malformations.

Group 3

Group 3 was described as 'conditions associated with immaturity (neonatal deaths only)'. It was decided that this group should be titled 'conditions associated with preterm birth or immaturity' and include only livebirths of under 37 weeks' gestation.

Infants weighing less than 1000 g should be presumed to belong to this group irrespective of the time of death. Larger preterm infants are likely to have suffered from birth asphyxia if they die at less than 4 hours of age. Thus any infant dying at less than 4 hours, delivered preterm, and weighing more than 1000 g, should be coded to group 4 below unless a specific condition was present.

Neonatal deaths with infection, even congenital infection, should be included here if they were delivered preterm, although specific infections—for example, group B streptococcus and toxoplasma, other viruses, rubella, cytomegalovirus, and herpes virus—should be coded group 5. The epidemiologist's view was that if some infections remained in group 3, then all infections should stay in group 3. The counter argument was that 'important and interesting' infections should go to group 5.

We came across problems with infants delivered at full term but who had disorders normally associated with prematurity such as hyaline membrane disease, intraventricular haemorrhage, and necrotising enterocolitis. It was decided that these should be coded as group 5.

Group 4

Group 4 in the original classification comprised 'asphyxial conditions developing in labour (fresh stillbirth/neonatal death)'. After discussion it was decided that this group should include all fetal deaths of whatever weight without malformations or specific disorders, provided that fetal death occurred during labour. In the absence of other information all fresh stillbirths should be included in this group.

Liveborn infants weighing over 1000 g who died at less than 4 hours of age should be included in this group. If the fetal death occurred during an intervention such as a caesarean section, in the absence of labour, the case should be classed group 4, as should any infants surviving longer than four hours for whom there was evidence of cerebral birth trauma or asphyxia. Massive antepartum haemorrhage such as abruption could form a clear subgroup.

Group 5

Group 5 was originally described as 'specific conditions other than above; and should include the following: blood group incompatibilities, inborn errors of metabolism, twin to twin transfusion syndrome, hydrops not associated with malformation, specific or unusual infections, conditions usually associated with preterm delivery but occurring in a baby born at full term, tumours, hamartomas, neonatal deaths of term babies that are totally unexplained, fetomaternal bleeds, or anything completely out of the ordinary.

The decision tree evolving from the refined classification is shown in the figure.

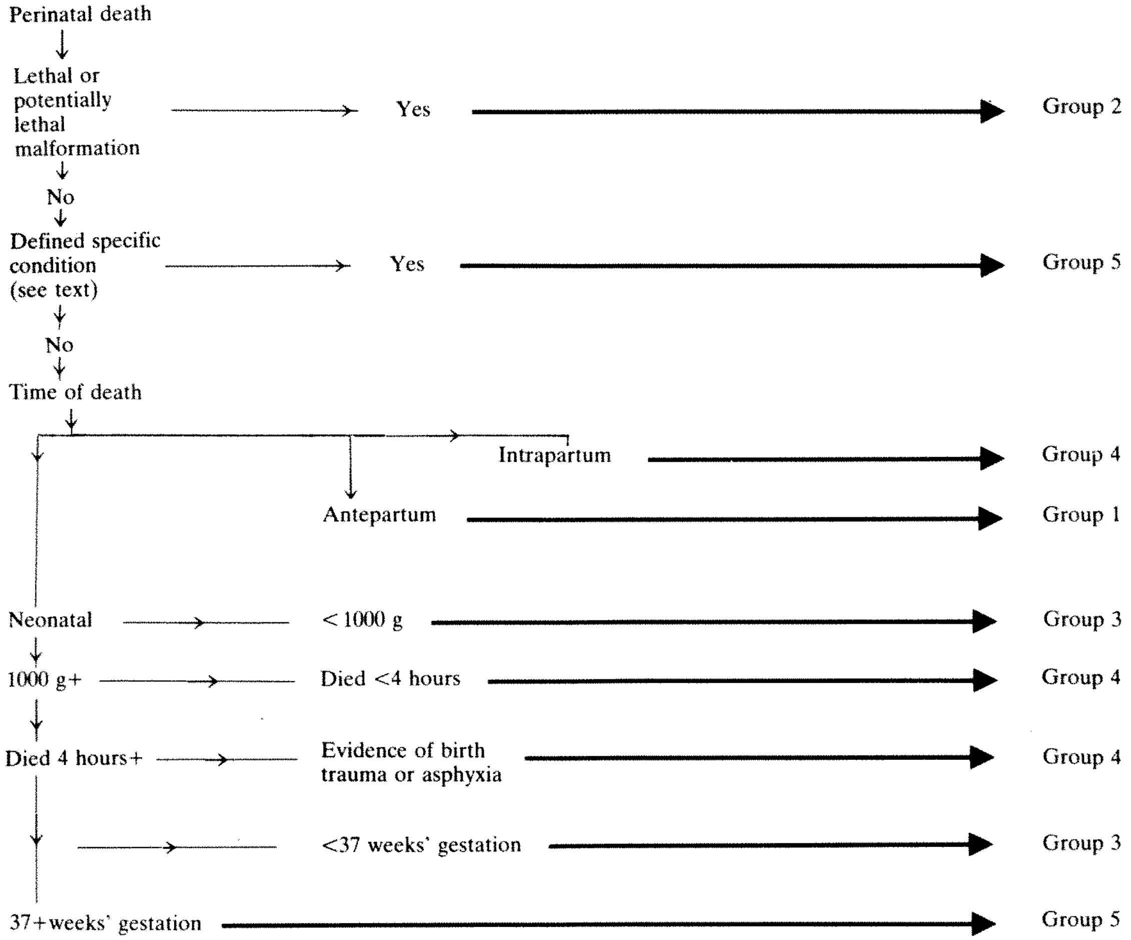

Figure *Decision tree for the revised Wigglesworth classification.*

Table 1 *Changes in Wigglesworth classification after necropsy and other findings had been reviewed*

Case No	*Classification*			*Comment*
	Clinical only	*Clinical plus necropsy*	*Clinical, plus necropsy, plus other findings*	
10	3	4	4	Tentorial tear
25	3	2	2	Congenital heart disease
117	3	2	2	Congenital heart disease
184	3	2	2	Congenital heart disease
65	1	2	2	Congenital heart disease
196	4	2	2	Congenital heart disease
123	1	2	2	Gastrointestinal defect
143	1	2	2	Congenital heart disease
137	2	5	5	Clinical congenital heart disease; at necropsy idiopathic arterial calcification
155	2	5	5	Congenital heart disease—collapsed during catheterisation; classified to iatrogenic
181	1	5	5	Idiopathic arterial calcification
151	4	4	5	Stormy labour, weighed 3580 g at 37 weeks, died at 7 hours. β haemolytic streptococcus grown
204	4	5	5	Weighed 3640 g at 40 weeks; died at 2 days of pneumonia
231	2	5	5	Antenatal scan showed cystic mass in neck; at necropsy non-rhesus hydrops found
175	4	5	4	At necropsy signs of sepsis found; on histological examination no sign of sepsis
97	3	3	5	β haemolytic streptococcus grown
100	1	1	5	Congenital cocksackie A virus isolated
145	1	1	5	Listeriosis
290	3	3	2	Trisomy 21
193	3	3	5	β haemolytic streptococcus grown
244	3	3	5	Enzyme defect

CHANGES IN THE CLASSIFICATION AFTER NECROPSY

Of the 239 cases considered, 14 (6%) were reclassified once the gross necropsy findings were considered (table 1) and it can be seen that most were because of the discovery of congenital anomalies at necropsy. In addition, consideration of other data including results of histological and microbiological examination further changed eight cases including one that had changed between the clinical to the gross necropsy classification (case 175). Interestingly, this case reverted to the classification that had been based on clinical grounds. Four of the other cases resulted from the identification of specific infections, one was an enzyme defect, and one a chromosome anomaly. Thus in all, 21 (8%) cases were reclassified as a result of necropsy and other findings. It is unlikely that 8% of diagnoses would be changed in geographic populations because the sample that we studied was not a representative series of perinatal deaths. It emanated from teaching hospitals with high rates of referral of complicated cases, and could therefore be expected to have a higher than normal proportion of unusual findings.

INTEROBSERVER RELIABILITY

The first sample of 89 cases were used as a learning exercise. The observers then compared results on the subsequent 144 cases in order to assess reproducibility. In all, for each set of case notes, there were 15 possible pairs of results. For each of the three assessments (clinical only, clinical together with gross necropsy, and clinical and all findings) there were 144×15×3—that is 6480 possible pairs.

Disagreement at initial classification was found for 963 pairs (15%). It should be noted that the 85% agreement rate is the interobserver variation and does not measure the accuracy of the classification. Major causes of disagreement concerned (i) whether a fetal death had actually occurred during labour, (ii) the class to which an immature infant should go, and (iii) how intrauterine infections should be classified. Despite agreement beforehand about definitions, clinical experience tended to override them.

PROBLEM CASES

Of the 233 cases considered there were 10 in which there was considerable discussion before eventual agreement. These are discussed below as examples of the most difficult cases to classify.

Case 16 was a diabetic cherub with visceromegaly who died before the onset of labour. The problem was whether this should be group 1 or 5; the ultimate decision was group 5.

Case 39 was a stillbirth that died before the onset of labour. Clinically it was recognised as having abnormal palmar creases and prominent heels, possibly as a result of a chromosome abnormality. At necropsy a solitary horseshoe kidney was found. The chromosome culture failed. This was eventually classified as group 2.

Case 51 was an infant born by caesarean section for severe pre-eclampsia at 26 weeks. The baby weighed 510 g and died aged 1 day. There was hydatiform change in the placenta. Should this be group 3 or 5? As this is an unexpected and unusual finding it was put into group 5.

Case 53: liquor had been draining for weeks since amniocentesis. The baby was born at 28 weeks' gestation, weighed 1160 g, and died at 90 minutes. Necropsy and histological examination confirmed a congenital infection but no specific organism was isolated. Various members of the team wanted to put it in group 3, 4, or 5. The rules, however, are clear and it was put into group 4.

Case 72 was an infant delivered at 28 weeks' gestation (1200 g) who died at 5 days. This was a breech extraction and the baby was clinically recognised as having aortic thrombosis with gangrene of the right toes and a flaccid left leg. Permission for a full necropsy was refused and external examination only was carried out. There was discussion about whether this should be classified to group 3, 4, or 5, but it was finally put in group 5.

Case 77 was a breech extraction at 27 weeks weighing 1271 g which died at 20 minutes. There had been prolonged rupture of the membranes with oligohydramnios and the infant had hypoplastic lungs. The rules put this into group 4.

Case 170 was born at 35 weeks' gestation weighing 1588 g. He had minor anomalies of the hands, limbs, and skull and died of massive pulmonary haemorrhage and disseminated intravascular coagulation at 7 days; this was put in group 3.

Case 186 was born at 37 weeks' gestation weighing 2330 g, and died at 4 hours of age. At necropsy the baby was found to have a polycystic right kidney, bilateral hydronephrosis and a double right ureter, and hypoplastic lungs. Gross necropsy examination showed a subarachnoid haemorrhage, hyaline membrane disease, and pneumonia. Further investigations identified mycoplasma pneumonia. The question was whether this should be classified as group 2, 3, 4, or 5. The team was divided, but the rules indicated group 5.

Case 212 was born at 36 weeks' gestation weighing 2240 g. It was dead at birth with mild hydrocephalus and undescended testes. Clinical notes stated clearly that death was before labour. Labour was of unknown duration. According to the rules this should have been coded as group 1 unless there was doubt as to whether the death occurred before or during labour.

Case 160 was born at 38 weeks' gestation weighing 2200 g and died at 4 days. There was cardiac malposition, a single umbilical artery, undescended testes, and anomalous pulmonary segments. The baby died of a massive pulmonary haemorrhage with a subarachnoid haemorrhage. There was much disagreement as to how this should be classified, but the final decision was that it should be group 5.

Table 2 *Aberdeen clinicopathological, and amended Wigglesworth, classifications after taking account of clinical and necropsy findings*

Aberdeen classification	*Wigglesworth classification*							*Total*
	1(a)	*1(b)*	*2*	*3*	*4(a)*	*4(b)*	*5*	
Premature, cause unknown	22	0	4	49	4	0	7	86
Mature, cause unknown	11	0	1	0	9	0	1	22
Mechanical	0	0	2	3	8	0	0	13
Toxaemia .	4	0	1	10	2	0	1	18
Antepartum haemorrhage	0	11	0	6	0	7	2	26
Malformation	0	0	43	0	0	0	3	46
Maternal disease	5	0	0	1	0	0	1	7
Rhesus incompatibility	0	0	0	0	0	0	6	6
Miscellaneous	0	0	0	0	0	0	4	4
Infection	0	0	0	1	0	0	4	5
Total	42	11	51	70	23	7	29	233

Subgroups (a) without and (b) with a history of abruption.

RELATION TO OBSTETRIC CLASSIFICATION

The way in which the Wigglesworth classification relates to the Aberdeen classification is shown in table 2. It is clear that the two are measuring different things. For example, of the 86 deaths classified by the Aberdeen rules as 'premature cause unknown' the Wigglesworth classification would distinguish the 22 dying before labour from the four dying of intrapartum causes, and the 11 dying of malformations or specific disorders from the 49 dying of disorders associated with immaturity.

Conversely, of the 30 that by the Wigglesworth classification would have been coded as intrapartum asphyxia, the Aberdeen classification would have distinguished the four 'premature—cause unknown' and the nine 'mature—cause unknown' from eight 'trauma' and two 'toxaemia'.

Discussion

As different observers become more aware of the usefulness of classifying perinatal deaths to compare performance over time and between centres, it is of importance to ensure that the classifications used are repeatable. A recent publication showed that the Aberdeen obstetric classification, which had been used for the Scottish National Survey and the Northern Regional Health Authority Survey, allowed differences in its interpretation.[10] These differences had developed both north and south of the border during the years since its introduction. The classification has been in use for 30 years and only in 1986 was a revised classification published with attention paid to current problems such as the death of the very immature neonate.[2] The authors concluded that different assessors can classify a series of deaths in a similar way, provided that close attention is paid to the definitions and working rules. They found that different groups of clinicians reached similar conclusions in 97% of cases submitted for assessment as long as they had a copy of the rules before them.

Nevertheless, there is considerable debate about the interpretation of all classifications of perinatal deaths. As most classifications used in the United Kingdom are based either on the Aberdeen or Wigglesworth classifications, we will confine our discussion to these two. Table 2 shows that, in general, the two classifications measure different things. Which is the more useful? Proponents of the Aberdeen classification suggest that it is important to know how many infants died from toxaemia, yet using that classification one is unlikely to find this out from the way the data are presented. The classification defines this group as having a diastolic pressure of 90 mm Hg or more on two separate days after 28 weeks' gestation together with appreciable proteinuria, and in the absence of hypertensive disease before pregnancy (which is comparatively common in pregnant women). Is it reasonable to assume that any normally formed baby born to a woman with this condition and dying prematurely is the result of toxaemia? Prevention of pre-eclampsia in the mothers may not have prevented most of these deaths. Similarly, the Aberdeen classification of 'trauma' (or under the revised classification, 'mechanical causes'), covered deaths from birth trauma and antepartum asphyxia associated with problems in labour such as disproportion, malpresentation, cord prolapse, cord compression, or breech delivery in babies of 1000 g or more. If there is no clinical history of difficulty in labour, but necropsy evidence of trauma is present, the deaths are not classified as 'mechanical causes' but as 'unexplained'. In other words, the trauma group does not indicate the number of deaths with evidence of birth trauma, but is a count of the number of deaths with abnormal presentation or method of delivery. It assumes that malpresentation is accompanied by asphyxia and is thus responsible for death. Malpresentation is, however, common in small babies, so this group might just be an indication of the proportion of preterm infants.

On the other hand, the Wigglesworth classification does not, in its primary analysis, examine causes of death in individual cases, so that subclassification within the primary groups may be necessary. The classification relies heavily on gestational age and time of death, and Wigglesworth has suggested that a number of assumptions can be made from these. A large number of deaths in group 1 (antepartum stillbirths) suggests that more attention to antenatal care might be warranted; many deaths in group 2 (congenital malformations) perhaps indicates that more attention should be given to preconceptional care; high numbers in group 3 (deaths associated with immaturity) might suggest that changes in neonatal intensive care were warranted; a high proportion of deaths in group 4 (intrapartum asphyxia) might indicate that obstetric care could be improved. It is always anticipated that group 5 will be a small group of miscellaneous causes from which few generalisations could be made, although it is a crude measure of the standard of perinatal histopathology, and other investigative services.

Since this study was carried out there has been much discussion among the participants. Each still prefers the classification that he has either developed or grown up with, but a majority were in favour of the revised Wigglesworth classification—perhaps because half the observers were perinatal pathologists. At the moment the topic of classification, especially the difference between the Aberdeen and the Wigglesworth classifications, is largely a British phenomenon. Nevertheless, publications from many countries are now appearing using the Wigglesworth classification, interpreting the results to emphasise areas in which medical care could be improved.[11-13]

Classification should be precisely defined so that it is easily applied by different people. In 10 cases (4%) no consensus was reached. This is too high, but most of these cases could have been classified by strict adherence to the revised rules.

Changes in classification occurred in 8% of cases when information derived from the necropsy and the results of all investigations were added to the clinical information. Though necropsy investigations contribute to the accuracy of classification, the extent of change is modest and is certainly acceptable when the alternative is omission of deaths not coming to necropsy. In most population studies this will be in excess of 30%.

Interobserver variability was 15%, and this degree of disagreement is too high for most studies. Scrutiny of the results showed that many discrepancies resulted from one person failing to apply the rules correctly, while 220 (23%) of the pairs of disagreement concerned the 10 cases that were resolved after further discussion.

We must reflect on the cause of the discrepancies. Is the classification insufficiently precise for universal application, or were the classifiers at fault? It is likely that the answer is the second possibility. When classification is undertaken by those with previous experience in a particular area it is likely that they will be swayed by their experience rather than adhering to guide lines, however precise. Possibly the answer would be to have the coding done by trained clerks rather than doctors, although there are cases (as we have seen) in which considerable judgment is required and it would be advisable in these doubtful cases to have a consensus decision from a medical panel.

References

[1] Baird D, Walker J, Thomson AM. The causes and prevention of stillbirths and first week deaths. *Br J Obstet Gynaecol* 1986;**93:** 1204–12.

[2] Whitfield CR, Smith NC, Cockburn F, Gibson AAM. Perinatally related wastage—a proposed classification of primary obstetric factors. *Br J Obstet Gynaecol* 1986;**93:**694–703.

[3] Claireaux AE. Perinatal mortality in the United Kingdom. In: Keller RJ, ed. *Modern trends in obstetrics III.* London: Butterworths, 1962:191–211.

[4] Naeye RL. Causes of perinatal mortality in the US Collaborative Perinatal Project. *JAMA* 1977;**238:**228–9.

[5] Wigglesworth J. Monitoring perinatal mortality: a pathophysiological approach. *Lancet* 1980;**ii:**684–6.

[6] Hey EN, Lloyd DJ, Wigglesworth JS. Classifying perinatal deaths: fetal and neonatal factors. *Br J Obstet Gynaecol* 1986;**93:**1213–23.

[7] Butler NR, Bonham DG. *Perinatal mortality. First report of the 1958 British Perinatal Mortality Survey.* Edinburgh: Churchill Livingstone, 1963.

[8] Dunn PM, Vulliamy CB. Perinatal mortality statistics of the Avon County population (UK), 1976–1985 using FIGO 1982 methodology. In: Kubli F, Patal N, Linderkamp O, eds. *Perinatal events and brain damage in surviving children.* Berlin: Springer-Verlag, 1987.

[9] Barson AJ, Tasker M, Lieberman BA, Hillier VF. Impact of improved perinatal care on the causes of death. *Arch Dis Child* 1984;**59:**199–207.

[10] Cole SK, Hey EN, Thomson AM. Classifying perinatal death: an obstetric approach. *Br J Obstet Gynaecol* 1986;**93:**1204–12.

[11] Tzoumaka-Bakoula C, Lekea V, McCarthy B, Golding J. Birthweight specific mortality in Greece. *Acta Paediatr Scand* 1989 (in press).

[12] Ashley D, McCaw-Binns A, Foster-Williams K. The perinatal morbidity and mortality study of Jamaica. *Paediatric and Perinatal Epidemiology* 1988;**2:**138–47.

[13] Sundhedsstyrelsen. Medicinsk fodsels-og misdannelsesstatistik 1986. *Vitalstatistik* 1988;**1:**23.

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Does mechanical ventilation precipitate gastro-oesophageal reflux during enteral feeding?

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SUMMARY The influence of intermittent positive pressure ventilation on gastro-oesophageal reflux in preterm infants is not known. In many neonatal units, however, concern that ventilation may increase gastro-oesophageal reflux (and therefore aspiration) leads to avoidance of enteral feeding during ventilation. We have therefore performed a crossover study of gastro-oesophageal reflux by monitoring lower oesophageal pH in a group of nine enterally fed, very low birthweight infants both during assisted ventilation and normal breathing. All infants had less reflux during intermittent positive pressure ventilation (mean (SEM) reflux index 2·3 (0·6%)) than during normal breathing (mean (SEM) reflux index 6·1 (1·1%)). Assisted ventilation was associated with a significant reduction in the gastro-oesophageal pressure gradient, an effect which may be related to the use of positive and end expiratory pressure during ventilation. These data show that fear of gastro-oesophageal reflux should not preclude the use of enteral feeding in preterm infants receiving ventilation.

Early and successful enteral nutrition of preterm infants has many advantages, including reduced reliance on parenteral nutrition[1] and the avoidance of its complications,[2] the emotional reward for mothers who provide milk for their babies, and the possibility of less osteopenia.[1] Infants who receive even small volumes of milk also show improved tolerance of feeds, reflecting a likely acceleration of gut maturation,[3] which may be mediated through the postcibal release of enteric hormones.[4] There are, however, possible disadvantages. During intermittent positive pressure ventilation (IPPV) preterm infants are said to be at high risk of gastro-oesophageal reflux, perhaps due to uncoordinated oesophageal peristalsis.[5] Regurgitated milk, once present in the oropharynx, may then be aspirated around a non-cuffed endotracheal tube.[6]

The association between enteral feeding, IPPV, and gastro-oesophageal reflux, however, has not previously been studied. This scarcity of evidence about the use of milk feeds during IPPV has led to wide variations in feeding policies. In a telephone survey of regional neonatal intensive care units we found that although in most units milk feeds were routinely introduced early during ventilation, in 36% of units milk feeds were seldom used during IPPV, and then only if long term ventilation was anticipated.

We have therefore performed a crossover study designed to determine the effect of IPPV upon gastro-oesophageal reflux in very low birthweight infants receiving intragastric feeds.

Subjects and methods

Nine infants were studied, all of whom were preterm (mean (SEM) gestation 27 (0·4) weeks), and of very low birth weight (1·04 (0·07) kg). During the study they were receiving at least 90 ml/kg/day of expressed breast milk as hourly bolus feeds through nasogastric tubes. Infants were closely observed by the nursing staff for signs of abdominal distension. The nasogastric tubes were aspirated four hourly before feeds, and feeds were only continued if the residual volume did not exceed 3 ml; most residual volumes were less than 1 ml.

Each infant was studied twice. In eight infants the first study was during IPPV, and the second while breathing spontaneously. The first study was performed at a mean (SEM) postconceptional age of 29 (0·5) weeks while receiving 123 (8) ml/kg/day of milk feed, and the second during normal breathing at a mean postconceptional age of 30 (0·3) weeks, while receiving 142 (8) ml/kg/day of milk feed. The differences in feed volumes were not significant.

The primary respiratory illnesses requiring IPPV were hyaline membrane disease (n=4), chest infection (n=2), congenital pneumonia (n=1), and chronic lung disease (n=2). Infants were ventilated on a time cycled, pressure limited ventilator, either the Drager Babylog (8403/300), or the Sechrist infant ventilator (100B). Positive end expiratory pressure of 3–5 cm H_2O was used for all infants on IPPV. During the study none of the infants was receiving sedation, muscle relaxants, or xanthine derivatives.

During the study infants continued to receive routine nursing care, which included chest physiotherapy for a few minutes carried out by a member of the nursing staff while the infant remained in the incubator. After physiotherapy mucus was aspirated from the oropharynx and endotracheal tube with a fine catheter. When necessary the nappy was changed in the incubator and a clean nappy loosely applied. There was no significant difference between the mean (SEM) number of episodes of nursing care during IPPV (13·1 (2·9) episodes/24 hours) and normal breathing 8·9 (2·2) episodes/24 hours. Infants were nursed in the prone, right lateral, and left lateral positions, and spent equal times in each position during both studies.

The protocol was approved by the district research ethics committee, and informed consent was obtained from the parents before each study.

A 1 mm monocrystalline antimony electrode (Monocrystant-infant, Synectics Medical) was used to monitor lower oesophageal pH continuously for 24 hours as previously described.[7] The pH electrode was bonded to a nasogastric tube (external diameter 1·2 mm) attached to a continuously perfused manometric system; this was used to identify the lower oesophageal sphincter and thereby position the nasogastric tube in the stomach with the pH electrode in the lower oesophagus 1–2 cm above the lower oesophageal sphincter. During the study infants were fed through the nasogastric tube and no change was made in their routine care.

The manometric system was used at the end of each study to measure resting lower oesophageal sphincter pressure as previously described.[8] Pressure measurements were made in eight of the infants during IPPV, and in all nine infants during normal breathing. The gastro-oesophageal pressure gradient (that is, the difference between the resting end expiratory pressure in the fundus of the stomach and in the body of the oesophagus) was then calculated.

Twenty four hour pH recordings were analysed, and the following variables were recorded: the number of episodes/24 hours; the number of episodes lasting longer than 5 minutes in every 24 hours; the reflux index % (the percentage of the total observation period during which the pH was less than 4) and the longest single episode (in minutes) in 24 hours. The reflux index was used as the simplest overall measure of the amount of reflux.[9]

Statistical analyses were performed by the paired Student's *t* test, and Wilcoxon's rank sum test. All means are shown with standard errors and, where comparisons are made, with the standard error of the mean of the differences (SE diff).

Results

Throughout the study the nine infants tolerated feeds well and there was no clinical evidence of gastrointestinal problems. In particular there was no vomiting and at no time was milk aspirated from the oropharynx.

During normal breathing there was significantly more gastro-oesophageal reflux than during ventilation, with an almost threefold increase in the reflux index from 2·3 (0·6) to 6·1 (1·1)% (SE diff 0·8,

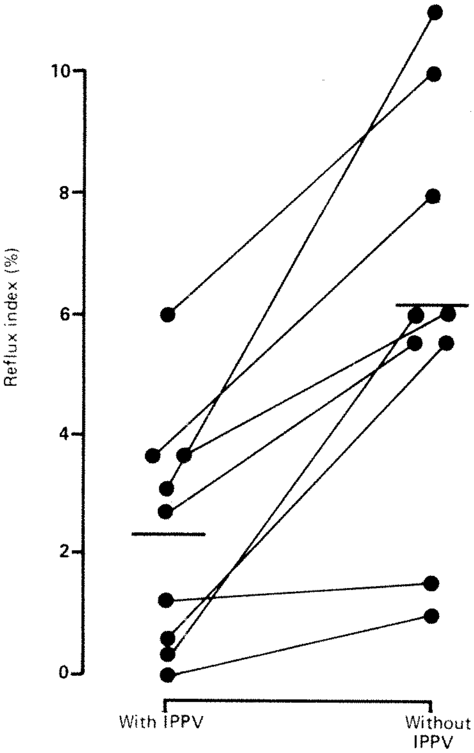

Fig 1 *Reflux index (%) in nine infants during assisted ventilation (with IPPV) and normal breathing (without IPPV). Means are shown as solid bars (p<0·01).*

Table *Gastro-oesophageal reflux and lower oesophageal sphincter pressures in nine infants during ventilation (IPPV) and normal breathing. Values are shown as mean (SEM)*

	Normal breathing	*IPPV*	*p Value*
No of episodes in 24 hours	19·4 (3·4)	12·2 (2·6)	>0·05
No of episodes lasting longer than 5 mins/24 hours	5·1 (1·2)	2·2 (0·9)	<0·05
Longest episode (mins)	14·7 (3·1)	7·2 (2·0)	<0·02
Reflux index (%)	6·1 (1·1)	2·3 (0·6)	<0·01
Resting lower oesophageal sphincter pressure (mm Hg)	8·4 (0·5)	7·0 (1·0)	>0·05

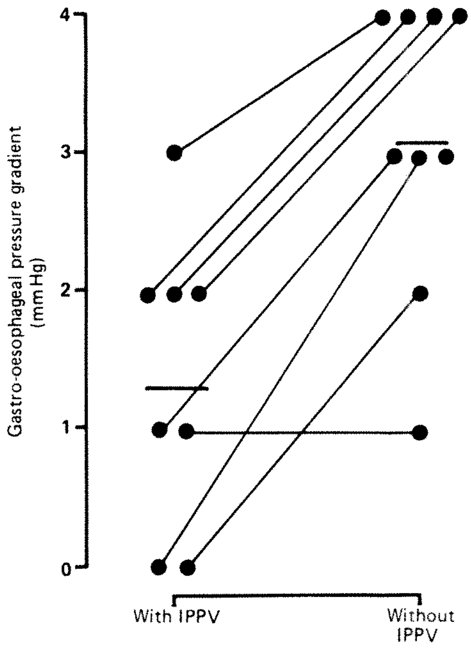

Fig 2 *Gastro-oesophageal pressure gradients in eight infants during assisted ventilation (with IPPV) and nine infants during normal breathing (without IPPV). ($p=0{\cdot}01$, Wilcoxon's rank sum test).*

$p<0{\cdot}01$) (fig 1). There were significant rises in the number of prolonged episodes (SE diff 1·3, $p<0{\cdot}05$), and the mean longest episode (SE diff 2·3, $p<0{\cdot}02$), although the increase in the total number of episodes did not achieve significance (table).

Oesophageal manometry showed no difference in mean lower oesophageal sphincter pressure between studies carried out during IPPV and normal breathing. The gastro-oesophageal pressure gradient during normal breathing, however (3·1 mm Hg, range 1–4), was significantly increased compared with that during IPPV (1·4, range 0–3, $p=0{\cdot}01$) (fig 2).

Discussion

In this crossover study of gastro-oesophageal reflux in infants receiving intermittent intragastric feeds, three times more gastro-oesophageal reflux and an increase in the gastro-oesophageal pressure gradients were observed during normal breathing compared with during assisted ventilation.

An early trial of feeding practice conducted in 1965 compared preterm infants on a regimen of early introduction of rapidly increasing volumes of milk feeds with those who had later introduction of smaller volumes of feed. A higher mortality was found in those on higher volumes of milk; necropsy studies suggested that this was caused by aspiration pneumonia.[10] Enteral feeds are still avoided in some units because of the fear of gastro-oesophageal reflux and subsequent pulmonary aspiration.[5 6 11] Indeed, in a recent textbook of neonatology it is stated that the early introduction of oral or nasogastric feeds in babies with respiratory distress syndrome is one of the commonest errors of management.[5] It is also stated, however, that the cautious early introduction of milk feeds in preterm infants not being ventilated is now common practice,[5] and we have found that in most regional neonatal intensive care units enteral feeds are now used during ventilation. In these units milk is introduced to avoid parenteral nutrition if possible, and with putative beneficial effects upon gut maturation[12] and motility,[3] and tolerance of feeds.[1]

Clearly the wide variation in attitude towards the use of enteral milk feeds among regional neonatal centres in the United Kingdom is compatible with the lack of compelling evidence for or against the safety of enteral feeding in preterm infants receiving IPPV.

We were surprised to find less gastro-oesophageal reflux during ventilation. During normal breathing the reflux index did not differ from our previously reported overall mean for the preterm, intensive care population.[7] Although we have previously shown that gastro-oesophageal reflux is increased after feeds and nursing care, there were no significant differences between these variables in the two study periods to explain the observed differences in gastro-oesophageal reflux. In fact, during IPPV the number of episodes of nursing care was marginally higher and endotracheal suction was also carried out, both of which might be expected to increase, rather than decrease, the amount of gastro-oesophageal reflux. Furthermore, the observed fall in the reflux index during ventilation was mainly the result of a reduction in the number of prolonged episodes of reflux. It is possible, therefore, that during IPPV either oesophageal clearance of gastric contents is improved, or that there is a reduction in the volume of gastro-oesophageal reflux.

The use of positive end expiratory pressure may be an important factor in the reduction of gastro-oesophageal reflux seen during IPPV. Positive end expiratory pressure results in positive intrathoracic pressure throughout the respiratory cycle and alters the physiological pressure difference between the abdominal and thoracic cavities. During normal breathing the resting pressure in the stomach exceeds that in the body of the oesophagus, thereby providing a positive gastro-oesophageal pressure gradient that predisposes towards reflux.[13] Constant reflux is prevented by, among other factors, the presence of the lower oesophageal sphincter. In this study the gastro-oesophageal pressure gradient during normal breathing was similar to that previously reported by us.[8] During IPPV, however, which included the use of positive end expiratory pressure, the gastro-oesophageal pressure gradient was significantly reduced ($p=0{\cdot}01$) (fig 2). The reduction in this pressure gradient, which predisposes to gastro-oesophageal reflux, may therefore help to explain our findings. It is noteworthy that we did not find any difference in resting lower oesophageal sphincter pressure between periods of IPPV and normal breathing.

Advice and practice for the optimal nutrition of preterm infants vary greatly, and it is clear that this area of clinical practice requires further critical evaluation. The data presented provide further evidence that enteral nutrition is not contraindicated during IPPV.

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References

[1] Dunn L, Hulman S, Weiner J, Kliegman R. Beneficial effects of early hypocaloric enteral feeding on neonatal gastrointestinal function: preliminary report of a randomized trial. *J Pediatr* 1988;**112**:622–9.
[2] Postuma R, Terence CL. Liver disease in infants receiving total parenteral nutrition. *Pediatrics* 1979;**63**:431–4.
[3] Bisset WM, Watt J, Rivers R, Milla P. The ontogeny of small intestinal motor activity. *Pediatr Res* 1986;**20**:692.
[4] Lucas A, Bloom SR, Aynsley Green A. The development of gut hormone response to feeding in neonates. *Arch Dis Child* 1980;**55**:678–82.
[5] Roberton NRC. Pulmonary disease in the newborn. In: Roberton NRC. *Text book of neonatology*. Edinburgh: Churchill Livingstone, 1986: 259–311.
[6] Goodwin SR, Graves SA, Haberkern CM. Aspiration in the intubated premature infant. *Pediatrics* 1985;**75**:85–8.
[7] Newell SJ, Morgan MEI, Durbin GM, Booth IW, McNeish AS. Gastro-oesophageal reflux in the preterm infant. *Arch Dis Child* 1989;**64**:780–6.
[8] Newell SJ, Sarkar PK, Durbin GM, Booth IW, McNeish AS. Maturation of the lower oesophageal sphincter in the pre-term baby. *Gut* 1988;**127**:1[illegible]7–72.
[9] Vandenplas Y, Sacre L. Continuous 24-hour oesophageal pH monitoring in 285 asymptomatic infants 0·15 months old. *J Pediatr Gastroenterol Nutr* 1987;**6**:220–4.
[10] Wharton BA, Bower BD. Immediate or later feeding for premature babies? A controlled trial. *Lancet* 1965;**ii**:969–72.
[11] Nussbaum E, Maggi JC, Mathis R, Galant SP. Association of lipid-laden alveolar macrophages and gastroesophageal reflux in children. *J Pediatr* 1987;**110**:190–4.
[12] Mayne AJ, Brown GA, Sule D, McNeish AS. The post-natal development of dissacharidase activities in the jejunal fluid of pre-term neonates. *Gut* 1986;**27**:1357–61.
[13] Cohen S, Harris LD. The lower oesophageal sphincter. *Gastroenterology* 1972;**63**:1056–73.

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Postprandial motor response of the small intestine to enteral feeds in preterm infants

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SUMMARY The postprandial motor response to enteral feeds of the small intestine of preterm infants is dependent on the bolus volume of feed that the infants receive and the number of days over which enteral feeds have been given. The postconceptional age of the infant is not an important determinant. The early introduction of enteral nutrition to preterm infants is therefore likely to enhance the motor response of the small intestine to feeds.

In the very preterm infant immaturity of gastrointestinal function frequently precludes successful postnatal adaptation, so that nutritional requirements cannot all be taken by the enteral route. For the efficient assimilation of luminal nutrients the integration of adequate digestive, absorptive, and motor function is required. Absorptive[1] and digestive[2] function are moderately well developed by 28 weeks' gestation, but the development of adequate motor function lags many weeks behind,[3] and thus limits tolerance to enteral feeds.[4] Motor function requires the integrated maturation of enteric nerves, endocrine cells, and smooth muscle cells.

Though enteral feeding enhances the development of intestinal humoral controls in the human infant,[5] and mucosal digestive function in animals,[6] little is known about the effect of feeding on the development of intestinal motor activity in preterm human infants.

Small intestinal motor activity can be divided into two distinct phases. Between meals a cyclical fasting or interdigestive pattern of motor activity occurs, which is disrupted after a meal and replaced by continuous activity that promotes mixing and segmentation of the luminal contents; the postprandial pattern (fig 1).

The fasting pattern in the child and the adult is characterised by the appearance about every 90 minutes of a highly organised band of phasic contractions that passes from the stomach along the small intestine to the ileum. The phasic contractions (phase III) are preceded by a period of irregular contractile activity (phase II) and followed by a period of quiescence (phase I); the whole recurring cycle is referred to as the migrating myoelectric or motor complex. Previous studies of fasting small intestinal motor activity in preterm infants have shown a clear gestationally dependent programme of maturation,[7] which is characterised by: low amplitude random contractions without obvious organisation (28–32 weeks' postconceptional age); short (four minute) bursts of organised contractile activity or clustered phasic activity (28–35 weeks); longer (12 minute) periods of propagated motor activity or prolonged phasic activity (34–36 weeks); and migrating motor complex activity occurring every 25 minutes (37–42 weeks).

The increase in the magnitude and the degree of organisation of fasting motor activity with increasing postconceptional age is probably related to the acquisition and development of enteric neural networks.

The postprandial pattern that develops after a meal normally lasts for 3–4 hours in the adult, at which time the fasting pattern returns. The aim of the present study was to define the longitudinal development of the motor response to food of infants born prematurely.

Subjects and methods

Nine preterm infants aged 28–42 postconceptional weeks, weight 830–3260 g, were studied, eight longitudinally (two subjects on four occasions, two on three occasions, and four on two occasions) and one on a single occasion (a total of 23 separate occasions). All but the most severely ill infants were included in the study and no infant was studied on more than four occasions. The study was approved by St Mary's Hospital district ethics committee.

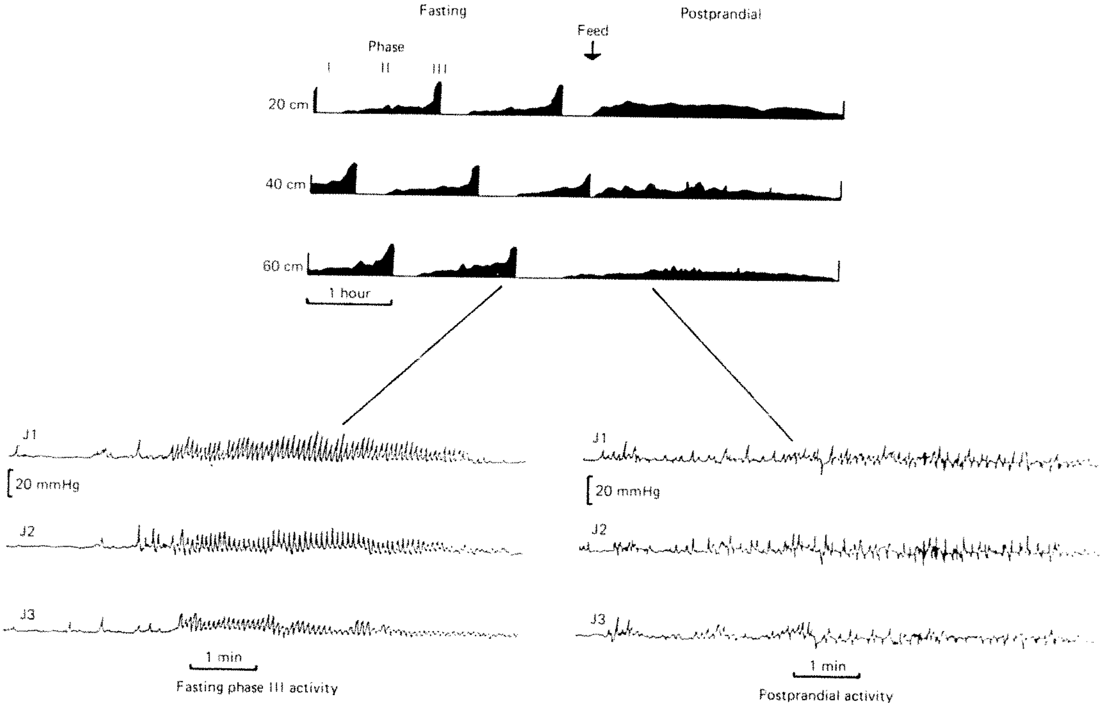

Fig 1 *A compressed recording of small intestinal motor activity from a 6 year old child with an expanded view of a period of fasting and postprandial activity from three different sites in the jejunum (J1, J2, J3) 5 cm apart.*

Informed consent was obtained from the parents of each infant before the study.

The infants' ages are given as the gestational age, that is the period of gestation at birth, or as the postconceptional age, which is the gestational age plus postnatal age.

Small intestinal motor activity was measured using a perfused catheter manometric technique. Soft polyvinyl chloride multilumen catheters external diameter 1·5 mm, internal diameter 0·7 mm (double channel) or 0·5 mm (triple channel) (Dural Plastics), were used in all studies. The distal perfusion ports were separated by 2·5 cm. In the first 13 studies the double lumen catheter was used. With the development of an improved technique of tube manufacture we were able to use a triple lumen catheter in the last 10 studies. The manometric catheter was perfused with sodium chloride 150 mmol/l, at a rate of 0·4 ml/channel/hour delivered by a syringe pump system (Harvard Infusion Pump). Pressure changes were measured by external Luerlock Mark 3 pressure transducers (Gaeltec), and the signal was displayed on a multichannel oscillographic chart recorder (Washington MD4).

The manometric catheter was advanced through the nose into the stomach of each subject and, while pressure changes were being recorded, further advancement through the pylorus and into the duodenum was monitored by the characteristic change in the frequency of motor activity from 3 cycles/min in the stomach to 12 cycles/min in the duodenum. In four of the smaller infants the position of the tube was confirmed by radiographic examination, which was carried out for clinical reasons. In each case the tip of the catheter was at the duodenojejunal flexure.

INFANT FEEDS

As we believed that it would be unethical to alter the management of the patients for the purpose of this study, the infants received a bolus feed equal to that which they were previously receiving, or (in those on continuous feeds) a volume equivalent to one hour's feed was given as a bolus. Some infants received expressed breast milk, some formula feed, and others received a mixture of the two. The energy density of all the feeds was similar. The very preterm infants received their feed nasogastrically and the more mature infants were fed orally. The nasogastric feeds were completed within 10 minutes and the oral feeds within 20 minutes. The recordings of small intestinal motility were made continuously both before, during, and after each feed. The study was designed to determine the effect of feeding on gut motor activity and not to compare one feed with another or to determine differences resulting from the mode of feed delivery.

ASSESSMENT OF MOTILITY

The infants were all fasted before the study. In most infants this was for between 4–24 hours, but in a few of the smaller infants, who were being totally enterally fed, only a two hour fast was possible if hypoglycaemia was to be prevented. During each study small intestinal motor activity was recorded for at least four hours. After an initial one hour period of fasting motor activity the infant was fed, as described above, and the pattern of postprandial motor activity was noted. The postprandial activity was characterised by the development of apparently random non-propagative activity that disrupted the basal fasting pattern. The length of postprandial activity was defined as the length of time taken from the beginning of a feed until the disappearance of the postprandial pattern and the return of the fasting pattern of activity.

The frequency of duodenal contractile activity during periods of continuous motor activity was measured by inspection of the manometric recording for each of the 23 studies.

STATISTICAL ANALYSIS

The association between variables is expressed by the correlation coefficient (r) and the significance of this association was derived from the F score by linear regressional analysis. Multiple regressional analysis was used to assess the significance of factors influencing the length of postprandial motor activity. Means are expressed plus or minus one standard deviation. The significance of the differences between normally distributed variables was tested by the paired Student's *t* test. Probabilities of 0·05 or less were regarded as significant.

Results

POSTPRANDIAL PATTERN

After feeding one of three postprandial responses occurred (fig 2):

(i) in the smallest infants (<31 weeks' postconceptional age) who were receiving low volumes of continuous enteral feed, postprandial activity did not occur and if a fasting pattern was seen this was not disrupted by the enteral feed.[7] Fig 2(a) shows a postprandial pressure recording from such an infant with clustered phasic activity and a complete lack of any postprandial response.

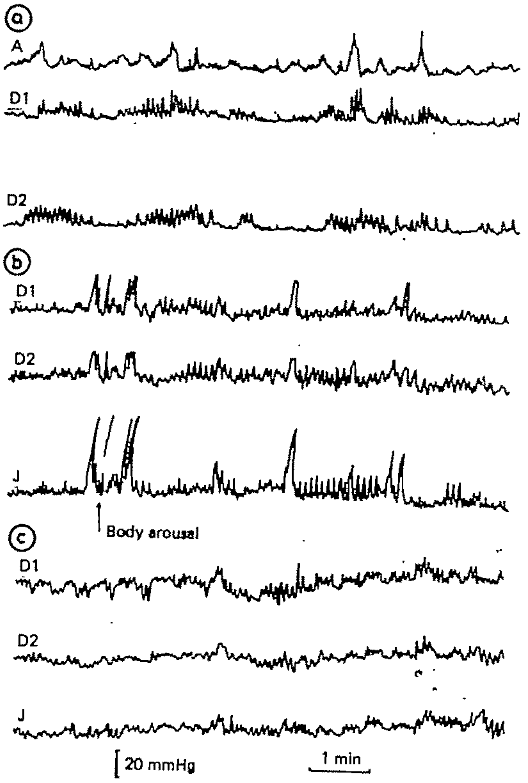

Fig 2 *Small intestinal motor activity recorded at multiple sites, within 15 minutes of the start of a bolus feed in infants of (a) 30 weeks' postconceptional age (b) 35 weeks, and (c) full term. The traces are recorded from A (antrum of stomach), D (duodenum), and J (jejunum).*

(ii) In infants of 31–35 weeks' postconceptional age, larger volumes of feed were given that induced some degree of postprandial activity, but often the underlying fasting pattern remained. In fig 2(b) a period of clustered phasic motor activity is superimposed on a background of more random postprandial activity.

(iii) Finally, in the older infants (>35 weeks' postconceptional age) who were receiving large volumes of bolus feed, there was complete disruption of the cyclical fasting activity with continuous postprandial activity lasting up to two hours as shown in fig 2(c). Towards the end of the postprandial period, however, fasting activity returned giving a pattern similar to that seen in the less mature infants.

FREQUENCY OF CONTRACTILE ACTIVITY

The mean frequency of maximum motor contraction in the duodenum estimated from all 23 recordings during the postprandial phase of activity of 11·5 (0·6) cycles/min was not significantly different from that found in the fasting state (11·4 (0·6) cycles/min).

MULTIPLE REGRESSION MODEL

A multiple regression model was developed to study the factors that might influence the postprandial response. The length of postprandial activity was used as the dependent variable and postconceptional age, bolus feed volume, and time fed enterally, as the independent variables. Fig 3 shows that with increasing postconceptional age the length of postprandial activity following a feed increased (r=0·6). This increase may, however, reflect the fact that the more mature infants were receiving larger volumes of feed and using the multiple regression model described above it can be seen that postconceptional age (p=0·37) contributes little, while the volume of feed (p=0·005) seems to be the most important determinant of the correlation. This is reflected in fig 4 where the length of postprandial activity plotted against bolus feed volume shows an improvement in the correlation coefficient with the length of activity from 0·6 to 0·78.

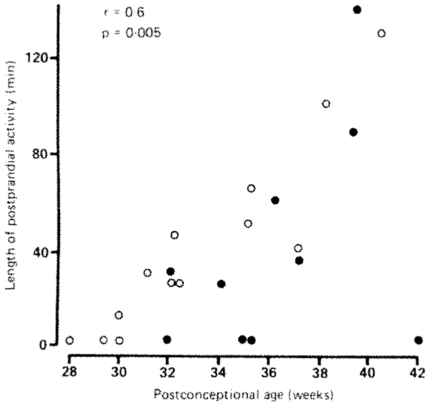

Fig 3 *The length of postprandial motor activity compared with the postconceptional age in 23 infants. Open circles denote those born at <29 weeks' gestation, and closed circles those born at ≥29 weeks.*

In both figs 3 and 4 those infants born before 29 weeks are differentiated from those born at 29 weeks or later. For a given postconceptional age (fig 3) those born more prematurely seemed to have better developed postprandial activity, but this difference is not significant (p=0·07). For a given feed volume, however (fig 4), those born more prematurely—and thus of greater postnatal age—have a significantly longer postprandial response (p=0·03). When the length of time that each infant had been on enteral feeds was plotted against the duration of postprandial activity (fig 5) an even stronger correlation between these variables was seen (r=0·89, p<0·0001). In addition, the previous differences between the two groups of preterm infants had disappeared. The length of postprandial activity also increased progressively from that observed in the continuously fed infants to that seen in those receiving four hourly bolus feeds (r=0·66, p=0·001). Thus the multiple regressional model clearly shows that the period of time during which

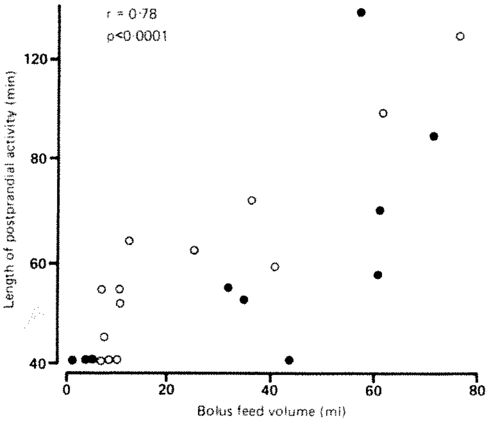

Fig 4 *The length of postprandial motor activity plotted against the volume of bolus feed in 23 infants. Open circles denote those born at <29 weeks' gestation, and closed circles those born at ≥29 weeks.*

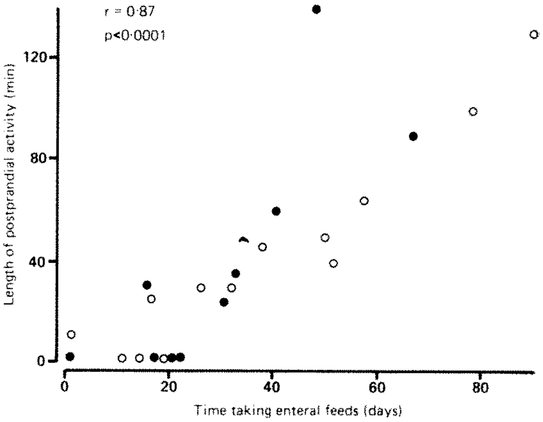

Fig 5 *Plot of the length of postprandial motor activity against the length of time on enteral feed in 23 infants. Open circles denote those born at <29 weeks' gestation, and closed circles those born at ≥29 weeks.*

Table *Correlation of variables with length of postprandial activity*

Variable	*Correlation*
Postconceptional age	0·60
Increasing feed interval	0·66
Postnatal age	0·78
Volume of bolus feed	0·78
Volume of daily feed	0·80
Time receiving enteral feeds	0·87

p Value <0·005 in all cases.

the infant has been taking enteral feeds and—to a lesser extent—the actual bolus feed volume, are important determinants of the postprandial motor response, while the postconceptional age of the infant seems to be of lesser importance. The factors that correlate with postprandial activity are summarised in the table.

Discussion

Unlike fasting motor activity, which appears to be tightly controlled by programmed actions of the enteric nervous system, postprandial activity represents the motor response of the gut to food and will vary according to the nature of the stimulus (the feed) and the ability of the gut to respond to that stimulus. Thus as the volume of enteral feed increases with increasing postconceptional age, so the degree of postprandial activity might also increase. Our data show that this is indeed the case, and from the analysis it would seem that the increased length of time taking enteral feeds and the increased feed volume are the principal determinants of this change, and postconceptional age is not important.

Disruption of fasting small intestinal motor activity and the induction of a postprandial pattern has been associated with the release of intestinal polypeptide hormones such as gastrin, cholecystokinin,[8] and peptide YY.[9] The magnitude and length of the postprandial motor response is associated with the volume and energy density of the feed delivered.[10 11]

If a certain degree of response is required to disrupt the fasting pattern of activity and initiate postprandial activity, this would account for the findings in the present study in infants receiving low volumes of feed in whom no postprandial response was seen. In those receiving larger volumes an intermediate response occurred with superimposed fasting and postprandial activity, and in those receiving large volumes of enteral feed there was complete disruption of the fasting pattern and replacement by postprandial activity for over an hour. The appearance of the intermediate response towards the end of a prolonged period of postprandial activity could result from the humoral response falling as the feed was digested and absorbed.

Our data show that the length of time that an infant has been taking enteral nutrition seems to be the most important factor in determining the length of postprandial activity. This suggests that the hormonal response that is responsible for the initiation of postprandial activity is enhanced by increased exposure of the gut to enteral nutrition. Support for this hypothesis is provided by previous studies in humans,[5 12] which show that the hormonal response to an enteral feed increased as the time taking enteral feeds increased. We did not record the cumulative volume of feed delivered to each of the infants as previous studies have shown that only low cumulative volumes (<96 ml) of feed are needed to produce a pronounced rise in the mean plasma concentrations of many polypeptide gut hormones, including some associated with postprandial activity, above the newborn concentrations.[13] The use of a factor combining length of time taking feed and feed volume, which might be most closely related to cumulative feed volume, did not increase the correlation with the length of postprandial activity above that seen using only length of time on feed. Though our data might suggest that there is an enhanced humoral response to enteral feeds related to the length of time that an infant has been fed, there are remarkably few data from studies which explore this possibility. Longitudinal studies are required to confirm that this is indeed the case.

The optimal feeding regimens for preterm infants continue to be debated. Our data suggest that as enteral feeding enhances the postprandial humoral response it should be started as early as possible. It is quite clear that total parenteral nutrition delays these beneficial effects.[5] It is possible that even small nutritionally insignificant volumes of feed could be of benefit to infants who could not tolerate full volume feeds.[13] The feeds may not only be beneficial in promoting villus absorptive[1] and digestive function,[6] but also in enhancing the humoral responses of the gut necessary for postprandial motor activity.[5 13] It might also be predicted that the type of feed and the mode of delivery of the feed will have some influence on the postprandial response. It is likely, however, that because of interindividual variations in this resonse, large numbers of infants would have to be studied to show significant differences between feeding regimens.

Thus while the development, of fasting motor activity in preterm infants is gestationally dependent and is related to the maturation of enteric and central neural connections,[7] the postprandial motor response in contrast seems to be mainly influenced by how much and for how long the infant has been fed. It would, therefore, seem that exposure of the gut to feeds enhances the humorally mediated postprandial response.

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References

[1] McNeish AS, Ducker DA, Warren IF, Davies DP, Harran MJ, Hughes CA. The influence of gestational age and size on the absorption of D-xylose and D-glucose from the small intestine of the human neonate. In: Elliott K, Whelan J, eds. *Development of mammalian absorptive processes.* Ciba Foundation Symposium 70. Amsterdam: Excerpta Medica, 1979:267–80.

[2] Grand RJ, Watkins JB, Torti FM. Development of the human gastrointestinal tract. *Gastroenterology* 1976;**70**:790–810.

[3] McLain CR. Amniography studies of the gastrointestinal motility of the human fetus. *Am J Obstet Gynecol* 1963;**86**: 1079–87.

[4] Dunn PM. Intestinal obstruction in the newborn with special reference to transient ileus associated with respiratory distress syndrome. *Arch Dis Child* 1963;**38**:459–67.

[5] Lucas A, Adrian TE, Christofides N, Bloom SR, Aynsley-Green A. Plasma motilin, gastrin, and enteroglucagon and feeding in the human newborn. *Arch Dis Child* 1980;**55**:673–7.

[6] Hughes CA, Dowling RH. Speed of onset of adaptive mucosal hypoplasia and hypofunction in the intestine of parentally fed rats. *Clin Sci* 1980;**59**:317–27.

[7] Bisset WM, Watt J, Rivers RPA, Milla PJ. The ontogeny of fasting small intestinal motor activity in the human infant. *Gut* 1988;**29**:483–8.

[8] Wingate DL, Pearce EA, Hutton M, Dand A, Thomson HH, Wunsch E. Quantitative comparison of the effects of cholecystokinin, secretin and pentagastrin on gastrointestinal myoelectric activity in the conscious fasted dog. *Gut* 1978;**19**: 593–601.

[9] Adrian TE, Smith HA, Calvert SA, Aynsley-Green A, Bloom SR. Elevated plasma peptide YY in human neonates and infants. *Pediatr Res* 1986;**20**:1225–7.

[10] Weisbrodt NW. Motility of the small intestine. In: Johnston LR, ed. *Physiology of the gastrointestinal tract.* Vol 1. New York: Raven Press, 1981:411–43.

[11] Amarnath RP, Berseth CL, Abell TL, Perrault J, Malagelada JR. The effect of nutrient concentration on small intestinal motility in preterm infants. *Gastroenterology* 1986;**91**:1043.

[12] Lucas A, Bloom SR, Aynsley-Green A. Development of gut hormone responses to feeding in neonates. *Arch Dis Child* 1980; **55**:678–82.

[13] Lucas A, Bloom SR, Aynsley-Green A. Gut hormones and 'Minimal enteral feeding'. *Acta Paediatr Scand* 1986;**75**:719–23.

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Early parenteral feeding of amino acids

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SUMMARY Serial 24 hour balance studies of nitrogen and energy were carried out over 10 days in two groups of ventilator dependent preterm infants, of comparable weight and gestational age. In one group (n=10) a parenteral amino acid source (Vamin 9) was started within 24 hours of birth, and in the other group (n=11) it was not started until 72 hours. The feeding protocol was otherwise identical. The nitrogen intake (286 compared with 21 mg/kg/day), energy intake (188 compared with 151 kJ), and nitrogen retention (120 compared with −133 mg/kg/day), were all significantly higher during the first three days of life in the group in which the amino acid solution was started early. There were no differences by 7–10 days. The early introduction of amino acids improves the early nutritional state of sick preterm infants.

Infants of less than 30 weeks' gestation are born at a time of otherwise rapid intrauterine brain and body growth. Unfed, these babies have limited energy reserves[1] and the rapid establishment of postnatal nutrition is essential if they are to survive intact.[2] A fetus of comparable postconceptional age is a good nutritional model for the preterm infant.[3] Using this model, nitrogen retention of about 320 mg/kg/day would be needed for the preterm infant of 24–34 weeks' postconceptional age (E Widdowson, personal communication).[4] Parenteral feeding with a nitrogen solution from birth would thus seem appropriate if this target is to be achieved and if early negative nitrogen balance is to be avoided.

Because of concerns about fibrin and casein hydrolysate protein solutions, many neonatal units continue to delay the introduction of an amino acid solution for several days after birth, as older solutions were associated with various metabolic complications including hyperammonaemia[5] and acidosis.[6] The more recent crystalline amino acid solutions have been modified to reduce these risks.

Preterm infants, particularly if they are unwell, are likely to require nutrients and energy in the immediate transitional period after birth at rates at least similar to those available in the uterus We aimed to find out if there was any increase in overall nitrogen retention and energy intake in sick infants of low birthweight who were ventilator dependent and who were given amino acids intravenously from birth.

Patients and methods

Ventilator dependent preterm infants of less than 30 weeks' gestation were entered sequentially on admission to the neonatal intensive care unit into one of two intravenous feeding regimen groups. Those in group E (early) were given intravenous glucose and nitrogen (as Vamin 9, KabiVitrum), within 24 hours of birth, while group L (late) initially received intravenous glucose alone, and nitrogen only after 72 hours of life. The nitrogen delivery rate in both groups was increased progressively over three days from 1 g/kg/day to a maximum of 3 g/kg/day. Intravenous fat (Intralipid, KabiVitrum) was introduced to both regimens at 7 days postnatal age. The infants continued to receive intravenous nutrition (based on standard clinical criteria) until oral feeding could safely be established. The feeding regimens of the two groups differed only in the time of introduction of amino acids. The vitamin intakes (for example, ascorbic acid) were similar in both groups. A computer based protocol was used for the prescription of parenteral solutions.

The criteria for inclusion in the study were that the infants completed serial 24 hour balance studies of nitrogen and energy over the first 10 days of life, and that at least 75% of their nitrogen and energy was delivered intravenously. Thirty two infants were initially recruited: 17 in group E and 15 in group L. One infant in group E and two in group L died within 72 hours of age; enteral feeding was established in the first few days in two infants in each group, and in four infants (three in group E and one in group L) urine collections were incomplete. Eleven infants in group E and 10 in group L therefore met all the study criteria for analysis.

Six of the 11 infants in group E had periods (up to 48 hours) when they could not receive protein solution—two on days 4 and 5 because of precipitation of the solution as a result of over prescription of calcium and phosphate; one on day 2, a second on day 3, and a third on day 5 because of pronounced hyperglycaemia and presumed infection; and the sixth infant for 48 hours on day 4 because of conjugated hyperbilirubinaemia. Two infants in group L did not receive protein for 24 hour periods—one on day 5 and one on day 6—because of precipitation of the solution.

All infants required intensive medical and nursing care support as defined by the British Paediatric Association criteria.[7] All infants were initially dependent on ventilators. Four of 11 in group E and two of 10 in group L developed pneumothoraces, one infant in each group developed post haemorrhagic hydrocephalus, two in each group developed metabolic acidosis (pH less than 7·20), four of 11 in group E and two of 10 in group L had a clinically important patent ductus arteriosus, and one of 11 and three of 10, respectively, developed systemic infection.

Serial metabolic balance studies of nitrogen and energy were carried out for 10 days. Timed 24 hour collections of urine and faeces were made; traditional markers could not be used because there was initially no enteral intake. Urine was collected from an attached bag with any leakage collected on preweighed disposable nappies, and faeces were collected into disposable one way nappy liners. Each 24 hour aliquot of urine and faeces was stored at −20°C until analysis. Nitrogen and energy content was analysed by Kjeldahl's technique adapted for small volumes, and ballistic bomb calorimetry, respectively.

Each infant was nursed in a conventional incubator in a thermoneutral environment during the study period. Fluid volume management was according to standard clinical practice. A record was kept of each 24 hours' (from the start of urine collection) parenteral and enteral nitrogen and energy intake.

Random assay checks of nitrogen and energy content of the parenteral solutions and milk feed given in the same 24 hour period were carried out to confirm data supplied by the pharmacy and the milk producer. Nude body weight was recorded daily on an electronic scale accurate to 0·5 g. Crown-heel length using a measuring board accurate to 0·5 mm and occipitofrontal head circumference were recorded weekly by a single observer (JS).

Blood was taken daily for routine electrolytes and biochemical analysis, and for plasma amino acids profiles on day 5 of the study. Amino acid analysis was by high performance liquid chromatography of orthophthalaldehyde derivatives of amino acids. Protein was removed from plasma with sulphasalicylic acid, adjusted to pH 9·5, and the amino acids reacted with orthophthalaldehyde. The resulting derivatives were separated by gradient high performance liquid chromatography on a reversed phase C18 column and detected fluorimetrically. The method has been extensively validated against other methods of analysis and gives reliable results (DA Reaveley, unpublished observations).

Statistical analysis was by the unpaired Student's *t* test and linear regression analysis, and the study was approved by the hospital ethics committee.

Results

At the time of entry to the study the two groups differed significantly only in the time of introduction of amino acids ($p<0·001$) (table 1). At 10 days, seven infants in group E were still ventilator and oxygen dependent, while 10 in group L were ventilator dependent and eight oxygen dependent.

The nitrogen and energy content of the parenteral solutions supplied by the pharmacy and any enteral milks used were within 2% of the amount that we intended.

The mean (SEM) 24 hour nitrogen retention for each infant over the study period is shown in fig 1. The nitrogen retention differed between the two groups in the first 72 hours of life ($p<0·001$). All infants in group E were in positive nitrogen balance throughout the study period with an overall mean (SEM) nitrogen retention of 122 (18) mg/kg/day. All infants in group L were in negative nitrogen balance during the first 72 hours of life with a mean (SEM)

Table 1 *Comparability of the two groups. Figures are mean (SEM)*

	Parenteral amino acid solution given		
	Within 24 h (n=11)	*At 72 h (n=10)*	*p Value*
Male:female	9:2	6:4	
Gestation (weeks)	28 (1)	28 (1)	NS
Birth weight (g)	1087 (95)	990 (78)	NS
Age of introduction of amino acids (hours)	23 (1)	78 (4)	<0·001
Supine length (cm)	36 (4)	36 (3)	NS
Head circumference (cm)	26 (2)	25 (2)	NS

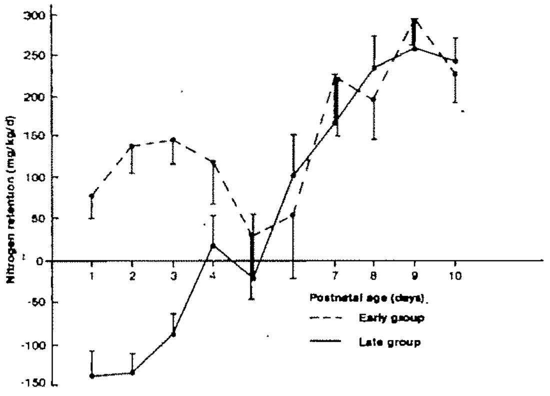

Fig 1 *Mean 24 hour nitrogen retention (mg/kg/d) for the two groups over the 10 day study period. Bars indicate SEM. Significant differences were recorded at days 1, 2, and 3 ($p<0\cdot001$). In the 'early group' the amino acid solution was given within 24 hours and in the 'late group' it was given at 72 hours.*

negative balance of −133 (23) mg/kg/day, compared with group E who retained 120 (6) mg/kg/day. After day 3 there was no difference in nitrogen retention between the two groups.

The total input and output balances over 10 days of intravenous and enteral intake and urine and faecal output are shown in table 2. There was no difference in fluid intake between the groups. In both groups a lower fluid intake was recorded in the first 24 hours of life compared with the remaining study period. The maximum mean (SEM) fluid intake in both groups was at 7 days postnatal age, being 149 (8) ml/kg/day in group E and 148 (8) ml/kg/day in group L. The mean percentage enteral energy intake of total energy intake at 10 days of age was 18% in group E and 24% in group L. There was no difference in the maximum mean (SEM) recorded urine outputs (both on day 5) of 111 (10) ml/kg/day, and 110 (13) ml/kg/day, in groups E and

Table 2 *Daily intake and output during the 10 day study period. Figures are mean (SEM)*

		Parenteral amino acid solution given	
		Within 24 h (n=10)	*At 72 h (n=11)*
Total fluid intake	(ml/kg/d)	126 (8)	126 (8)
Enteral intake	(ml/kg/d)	9 (3)	11 (4)
Urine output	(ml/kg/d)	85 (5)	97 (3)
Faecal output	(g/kg/d)	0·5 (0·1)	0·7 (0·3)

There were no significant differences between the groups.

Table 3 *Daily energy and nitrogen intakes given as mean (SEM), and energy:protein ratio (%) in the two groups*

	Days	*Parenteral amino acid solution given*		
		Within 24 h (n=11)	*At 72 h (n=10)*	*p Value*
Energy (kJ/kg/d)	1–3	188 (13)	151 (4)	0·001
	4–6	264 (13)	268 (13)	NS
	7–9	297 (13)	330 (17)	0·05
Nitrogen (mg/kg/d)	1–3	286 (15)	21 (6)	0·001
	4–6	286 (50)	232 (19)	NS
	7–9	412 (34)	423 (24)	NS
Energy:protein ratio (%)	1–3	17	1*	
	4–6	12	10	
	7–9	15	13	

*Refers to day 3 only.

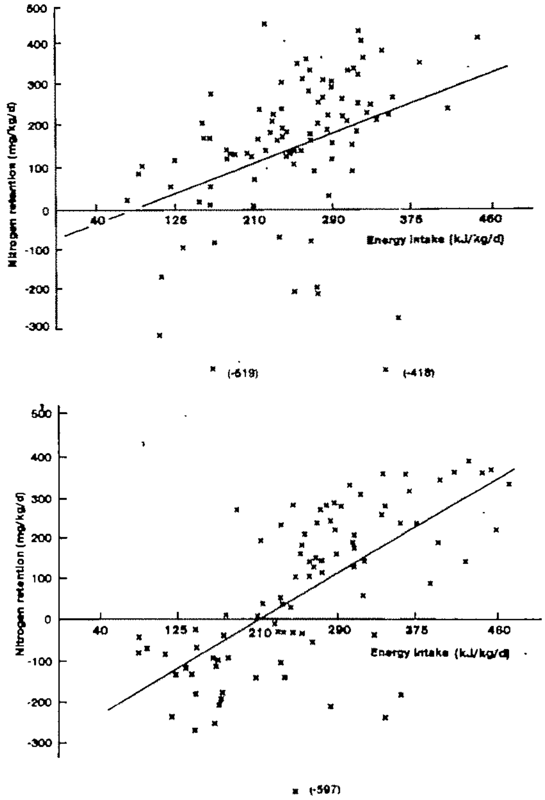

Fig 2 *Energy intake (kJ/kg/d) and nitrogen retention (mg/kg/d) for the group in which the amino acid solution was given within 24 hours (above) ($r=0\cdot35$, $p<0\cdot001$), and for that in which it was given at 72 hours (below) ($r=0\cdot66$, $p<0\cdot001$). Each point represents a 24 hour balance period.*

L, respectively. Faecal output was less than 0·7 g/kg/day in both groups throughout the study.

The mean daily nitrogen (mg/kg/day) and energy (kJ/kg/day) intake expressed in 72 hour periods is given in table 3. Nitrogen and energy intake was greater in group E in the first 72 hours ($p<0·001$); mean energy intake was greater in group L at 7–9 days ($p<0·05$). In group L protein intake contributed less than 2% of the overall energy intake in the first 72 hours. Otherwise the protein:energy ratio was greater than 10% in both groups throughout the study (table 3).

The association between mean daily nitrogen retention (mg/kg/day) and energy intake (kJ/kg/day) is shown in fig 2; there was a positive correlation in both groups ($r=0·35$, $p<0·001$ in group E; $r=0·66$, $p<0·001$ in group L). There was a wide range of nitrogen retention for individual infants at any given energy intake. Energy intakes of greater than 418 kJ/kg/day were achieved in only seven 24 hour balance periods, one in group E, and six in group L. The percentage of total nitrogen intake retained on days 1–3, 4–6, and 7–9 postnatal age was 42, 18, and 60% in group E, and 0, 22, and 51% in group L, respectively.

The increases in length, occipitofrontal head circumference, and total change in body weight were similar in both groups. Overall weight change was not significantly different in either group (table 4). Group E had regained 95% and group L 93% of their birth weights by 10 days of age. The age of maximum weight loss was day 6 postnatal age in group E, with 10% loss from birth weight, and day 7 in group L with 12%. There was no association between increase in length or head circumference, and energy or nitrogen intake, in either group.

Plasma aminograms at 5 days of age were available only for nine infants in group E and seven in group L. A high phenylalanine concentration (greater than 300 μmol/l) was found in two infants in group E and none in group L. Five infants in group E and four in group L had plasma tyrosine concentrations greater than 300 μmol/l. Other amino acids were within the laboratory reference range for neonates.

Table 4 *Changes in weight, occipitofrontal head circumference, and crown-heel length in the two groups. Figures are mean (SEM)*

	Days	*Parenteral amino acid solution given*	
		Within 24 h (n=11)	*At 72 h (n=10)*
Weight change (g/kg/d)	1–3	−23 (10)	−23 (6)
	4–6	−10 (16)	−8 (21)
	7–9	−3 (14)	−17 (14)
Occipitofrontal head circumference (cm/week)*	1–10	0·4 (0·1)	0·4 (0·1)
Crown-heel length (cm/week)	1–10	0·7 (0·1)	0·7 (0·2)

*Excluding one patient from each group with hydrocephalus.

Discussion

There is no general agreement on the ideal age at which to introduce an intravenous nitrogen solution to the feeding regimen of a preterm infant. In the infant who can be fed enterally, a protein source (milk) is introduced within hours of birth. These babies are usually well and often larger than the babies studied here. We would suggest that there is no reason why the sick infant requiring intravenous feeding should not be offered amino acids from day 1.

In this study infants in the late introduction group had a significant negative nitrogen balance until intravenous nitrogen was introduced on day 3. The mean negative balance of −133 mg/kg/day is equivalent to a loss of 0·849 g/kg/day of body protein, or 2·5 g protein over the first 3 postnatal days. The total body protein of an infant weighing 1000 g is about 85 g, and therefore assuming that the overall nitrogen (protein) loss represents true tissue catabolism then this represents a mean accumulative loss of 3% of the body's protein each day.[4] Infants in the early group, on the other hand, were in positive nitrogen balance from day 1 postnatal age.

It is likely that the overall positive nitrogen balance would probably have been greater if all infants in the early study group had received amino acid without interruption in the first few days. Although it is uncertain whether there is a long term clinical advantage in this early positive nitrogen balance, it may be reasonable to assume such an advantage, particularly during the critical transitional phase of extrauterine adaptation. It must be noted, however, that although the infants in the early group were in positive nitrogen balance from day 1, the mean daily nitrogen retention rate of both groups only reached the equivalent postconceptional age matched fetal accretion rate after day 7.[4 8]

In neither group were we able to achieve an energy intake greater than 209 kJ/day in the immediate postnatal period, which we recognise is considerably below current recommendations for enterally fed infants. In the early group, however, the overall energy intake was significantly higher than in the late group. The inability to achieve a critical energy threshold to maintain basal metabolism might partly explain the poor early growth seen in sick infants. The protein:energy ratio in all infants in group E, and after three days in Group L, was satisfactory. This implies that the energy supplied was adequate to permit the amino acids to be used.

We are currently studying the metabolic effects associated with the introduction of Intralipid from day 1. Intralipid has a high energy density; early introduction might permit a further increase in energy intake in these infants and improve the nitrogen sparing effect of energy.

Changes in body weight and gains in crown heel length and head circumference were similar in both groups. The mean negative changes in body weight observed in the first few days in both groups could partly be explained by shifts in body water,[9] and were similar to changes reported by others.[10] Enterally fed low birthweight infants show increased growth rates with protein intakes of 3·5 g/kg/day.[11 12] In our study a mean protein intake of 1·8 g/kg/day was achieved in the first few days after the early introduction of amino acids and this is likely to have been insufficient to cause an early increase in growth. Metabolic complications precluded any further increase in protein load.

Increased phenylalanine and tyrosine concentrations were seen in some infants in both groups. Abnormalities in plasma aminograms, and in particular increased phenylalanine concentrations, are well recognised in preterm infants given Vamin as the amino acid solution[13 14]; we did not see any obvious short term metabolic or other problems, but the long term implications are unknown. The introduction of the newer parenteral solutions may prevent these abnormalities. Whether breast milk or fetal lean body protein is the ideal nutritional source model for these infants, however, remains to be investigated.

A reasonable nutritional goal in feeding preterm infants is to approximate the nutritional accretion and growth seen in the fetus of comparable postconceptional age. A secondary aim in the immediate newborn period is to prevent significant catabolism and negative nitrogen balance.[2] The present study shows that early introduction of an intravenous amino acid source indeed permits positive nitrogen balance in the first days of life in the sick ventilator dependent infant who cannot be fed enterally.

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References

[1] Heird WC, Driscoll JM Jr, Shullinger JN, Grebin B, Winters RW. Intravenous alimentation in paediatric patients. *J Pediatr* 1972;**80**:351–72.

[2] Adamkin DH. Nutrition in the very low birthweight infants. *Clin Perinatol* 1986;**13**:419–43.

[3] American Academy of Pediatrics. Nutritional needs of low birthweight infants. *Pediatrics* 1985;**75**:976–86.

[4] Shaw JCL. Parenteral nutrition in the management of sick low birthweight infants. *Pediatr Clin North Am* 1973;**20**:333–57.

[5] Johnson JD, Alberitton WL, Sunshine P. Hyperammonaemia accompanying parenteral nutrition in newborn infants. *J Pediatr* 1972;**81**:154–61.

[6] Heird WC, Dell RB, Driscoll JM, Grebin B, Winters RW. Metabolic acidosis resulting from intravenous alimentation mixtures containing synthetic amino acids. *N Engl J Med* 1972;**287**:943–8.

[7] British Paediatric Association. Categories of babies requiring neonatal care. *Arch Dis Child* 1985;**60**:559–601.

[8] Kovar IZ, Saini JR, Morgan JB. The sick very low birthweight infant fed by parenteral nutrition: studies of nitrogen and energy. *European Journal of Clinical Nutrition* 1989;**43**:339–46.

[9] Meurling S, Arturson G, Zaar B, Eriksson G. Energy, fat and nitrogen balance in healthy newborn infants during the first weeks after birth. *Acta Chir Scand* 1981;**147**:487–95.

[10] Yu VYH, James B, Hendry P, MacMahon RA. Total parenteral nutrition in very low birthweight infants: a controlled trial. *Arch Dis Child* 1979;**54**:653–61.

[11] Kashyap S, Forsyth M, Zucker C, Ramakrishan R, Dell RB, Heird WC. Effects of varying protein and energy intakes on growth and metabolic response in low birthweight infants. *J Pediatr* 1986;**100**:955–63.

[12] Reichman BL, Chessex P, Putet G, *et al*. Partition of energy metabolism and energy cost of growth in the very low birthweight infant. *Pediatrics* 1982;**69**:446–51.

[13] Puntis JW, Edwards MA, Green A, Morgan I, Booth IW, Ball PA. Hyperphenylalanaemia in parenterally fed newborn babies. *Lancet* 1986;**ii**:1105–6.

[14] Evans SJ, Wyne-Williams TCJE, Russell CA, Fairbrother A. Hyperphenylalanaemia in parenterally fed newborn babies. *Lancet* 1982;**ii**:1404–5.

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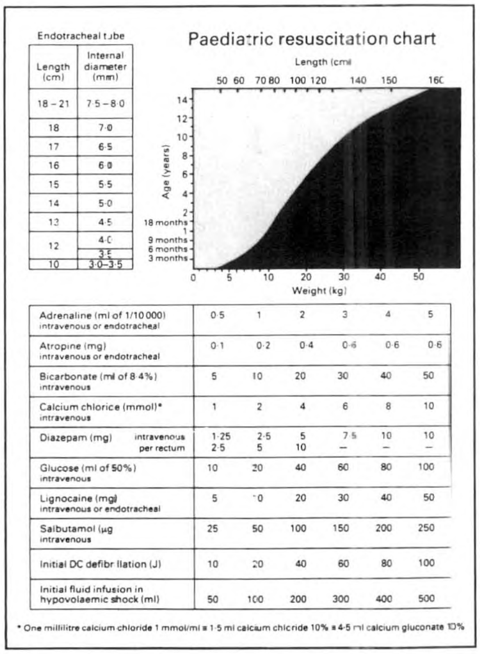

Endotracheal tube

Length (cm)	Internal diameter (mm)
18 – 21	7·5 – 8·0
18	7·0
17	6·5
16	6·0
15	5·5
14	5·0
13	4·5
12	4·0 3·5
10	3·0–3·5

Adrenaline (ml of 1/10 000) intravenous or endotracheal	0·5	1	2	3	4	5
Atropine (mg) intravenous or endotracheal	0·1	0·2	0·4	0·6	0·6	0·6
Bicarbonate (ml of 8·4%) intravenous	5	10	20	30	40	50
Calcium chloride (mmol)* intravenous	1	2	4	6	8	10
Diazepam (mg) intravenous per rectum	1·25 2·5	2·5 5	5 10	7·5 —	10 —	10 —
Glucose (ml of 50%) intravenous	10	20	40	60	80	100
Lignocaine (mg) intravenous or endotracheal	5	10	20	30	40	50
Salbutamol (µg intravenous	25	50	100	150	200	250
Initial DC defibrillation (J)	10	20	40	60	80	100
Initial fluid infusion in hypovolaemic shock (ml)	50	100	200	300	400	500

* One millilitre calcium chloride 1 mmol/ml ≡ 1·5 ml calcium chloride 10% ≡ 4·5 ml calcium gluconate 10%

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Carbohydrate fermentation by gut microflora in preterm neonates

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SUMMARY To study organic acid excretion, urine was collected from 52 preterm infants at weekly intervals and analysed by capillary gas chromatography-mass spectrometry. Twelve of 22 babies born before 33 weeks' gestation excreted 2,3-butanediol, as did six born between 33 and 36 weeks. Six very immature babies also excreted acetoin, the metabolic precursor of the diol. Other products derived from carbohydrate included methylmalonic and ethylmalonic acids in one baby, and D-lactic acid in five. Acetoin has never been found in urine before, and the other four acids have been found only rarely. Excretion of these metabolites by preterm babies can be explained by increased intestinal permeability, unabsorbed lactose in the colon, and colonisation with certain opportunistic micro-organisms prevalent in neonatal units, including klebsiella, serratia, and enterobacter. The findings support evidence from breath hydrogen analysis that carbohydrate fermentation takes place in the gut of preterm infants.

The colonic microflora can ferment carbohydrates, producing a range of organic acids[1] that may be absorbed from the intestine.[2] Some undergo further metabolism by the host[3,4] and may provide an important source of energy,[2] particularly for premature babies.[5,6] These acids cannot normally be detected in urine by conventional gas chromatography techniques. Certain carbohydrate fermentation products may become detectable in rare circumstances, however, when the gut physiology is disturbed. For example, D-lactic acid excretion has been reported in preterm babies with necrotising enterocolitis,[7] and in older subjects following extensive ileal resection when large amounts of unabsorbed carbohydrate in the colon were metabolised by lactobacilli.[8,9] Transient methylmalonic aciduria in a child with Hirchsprung's disease was attributed to gut bacterial activity.[10] Finally, two acidotic babies without an inherited metabolic defect were found to excrete 2,3-butanediol, an abnormal metabolite that was probably also derived from bacterial metabolism.[11]

A study was undertaken to investigate the organic acid excretion of premature babies. An unexpected finding was that products of bacterial carbohydrate fermentation were frequently detected in samples from babies born before 33 weeks' gestation.

Patients and methods

Fifty two preterm babies admitted to the special care baby unit of the Princess Anne Hospital, Southampton, from June to November 1985 were studied during their normal clinical management for up to 83 days of life. For the purpose of analysis, study patients were divided into two groups: 22

Table 1 *Details of infants studied*

	Group 1 (<33 weeks' gestation)	*Group 2 (33–36 weeks' gestation)*
No of babies	22	30
Median (range) gestation (weeks)	28 (25–32)	34 (33–36)
Median (range) birth weight (g)	1160 (730–1950)	2155 (1400–2670)
Median (range) No of days monitored	37 (1–83)	17 (0–29)
Total No of urine samples taken	127	89
No of samples taken after age 7 days	109	51
Mean (range) creatinine concentration (mmol/l) after age 7 days	1·12 (0·15–3·60)	1·01 (0·10–2·23)

babies born before 33 weeks' gestation (group 1), and 30 born between 33 and 36 weeks' gestation (group 2) (table 1). Nineteen babies in group 1 (86%) and two in group 2 (7%) were of very low birth weight (VLBW: birth weight less than 1500 g), and five babies in group 1 weighed less than 1000 g. Two babies in group 1, and 11 in group 2, were classed as small for gestational age, having a birth weight below the 10th centile for gestation.

Random urine samples were collected into a bag, once during the first 48 hours of life and then at weekly intervals until discharge. Samples were frozen immediately and stored at −20°C without preservative.

For the first 72 hours the babies born before 33 weeks' gestation received dextrose intravenously. They were then fed parenterally until early respiratory and circulatory problems were overcome (generally up to 10–20 days of age). The full regimen was established over five days and comprised Vamin 9 glucose, 10% Intralipid, Ped-el, Vitlipid, and Solivito (all KabiVitrum), and 10% dextrose. Oral feeding with expressed breast milk was introduced as the condition improved. Other milks were often substituted later and sometimes included a low birth weight formula (LBW Gold Cap SMA, Wyeth). Daily supplements of iron, 1α-cholecalciferol, and vitamins (Dalivit, Paines, and Byrne) were introduced from around 21 days. Parenteral nutrition was reintroduced if necrotising enterocolitis developed. The carbohydrate present in human milk and the standard formula feeds used was lactose, 12·6 to 13·1 g being provided by a daily intake of 180 ml/kg.[12] Low birth weight formula (SMA) contains lactose and maltodextrin (1:1), 180 ml/kg daily providing 7·74 g of each sugar. Babies of 33 to 36 weeks' gestation sometimes required dextrose intravenously in the first days of life, but were then fed orally on breast milk or formula feeds. Low birth weight formula (SMA) was given infrequently. None received parenteral nutrition.

Chemicals for analysis were obtained from either Sigma or Aldrich. Urine samples were tested for phenylketones, reducing substances and other abnormalities using Phenistix, Clinitest and Multistix, respectively, all from Ames, Miles Laboratories. Creatinine was measured by an alkaline picrate method. After oximation with hydroxylamine hydrochloride, urinary organic acids were extracted with ethyl acetate and diethyl ether, derivatised, and analysed by capillary gas chromatography. Unknown compounds were identified by combined gas chromatography-mass spectrometry.[13] To enable comparisons, acids were quantified from their peak areas using n-tetracosane as internal standard and the concentrations related to urinary creatinine. Babies within each study group were of similar gestational age, chronological age, and birth weight, and after 7 days of life their urinary creatinine concentrations fell within a narrow range (table 1). Errors from the use of creatinine ratios are unlikely to have influenced the findings significantly. D-lactic acid was analysed kinetically by its conversion to pyruvate by D-lactate dehydrogenase (from *Leuconostoc mesenteroides*, Sigma) by a method based on that of Gawehn and Bergmeyer.[14]

Limited information about the faecal coliform organisms was available retrospectively for seven babies who were also recruited into a microbiological study. Rectal swabs were cultured aerobically on blood agar and MacConkey's medium. Coliform bacteria were identified using a commercial system (Mast Diagnostics). No attempt was made to isolate anaerobes.

The χ^2 test was used to assess the significance of differences between groups. The study was approved by the local district ethics committee.

Results

URINARY EXCRETION OF 2,3-BUTANEDIOL AND ACETOIN

Three unknown peaks were seen in the organic acid

Table 2 *Metabolites derived from carbohydrates by bacteria detected in 216 urine samples*

Metabolite	*No of babies who excreted metabolite*	*No (%) positive samples*	*Median (range) metabolite concentration (mg/mmol creatinine)*
Gestational age <33 weeks (127 samples from 22 babies):			
2,3-Butanediol	12	36 (28)	17·4 (0·8–378·4)
Acetoin	6	18 (14)	3·6 (trace–18·6)
Methylmalonic acid	1	4 (3)	4·6 (4·1–10·8)
Ethylmalonic acid	1	3 (2)	1·5 (1·33–1·60)
Gestational age 33–36 weeks (89 samples from 30 babies):			
2,3-Butanediol	6	10 (11)	3·6 (0·6–17·0)
Acetoin	0	0	
Methylmalonic acid	0	0	
Ethylmalonic acid	0	0	

profiles of some samples. Two of these were found to be isomers of the sugar metabolite 2,3-butanediol. The third peak was usually seen when large amounts of diol were present and was shown to be its metabolic precursor, acetoin.[13] Acetoin was never seen in the absence of 2,3-butanediol. Neither compound was present in any of the milks, vitamins, or intravenous preparations given.

2,3-Butanediol was present in 46 of the total of 216 samples (21%) (table 2). The mesoisomer predominated, and in 21 samples was the only form present. More babies born before 33 weeks excreted the diol (12 of 22, 55%) than those of 33 to 36 weeks' gestation (six of 30, 20%; $p<0.01$), and acetoin excretion was confined to this group. The concentrations of 2,3-butanediol detected were also higher, exceeding 10 mg/mmol creatinine in seven very immature babies (31, 32, 71, 139, 224, 351, and 378 mg/mmol creatinine) compared with only two in the 33–36 week group (15 and 17 mg/mmol creati-

Table 3 *Excretion of bacterial metabolites related to feeding and antibiotic treatment*

Case No	*Gestation (weeks)*	*Birth weight (g)*	*No of positive samples*	*At time abnormality diagnosed*				*Antibiotics given before abnormality diagnosed*
				Age (days)	*Feed being given*	*Days on oral feeds*	*Days off antibiotics*	
2,3-Butanediol with or without acetoin:								
1	25	800	1	13	Total parenteral nutrition, plus expressed breast milk for 24 hours	1	0	Benzylpenicillin, cefotaxime; in progress: gentamicin and flucloxacillin
2	26	940	6	25–57	Expressed breast milk	6	6	Benzylpenicillin, cefotaxime, and flucloxacillin, amikacin, and fucidin
3	26	862	4	30–64	Expressed breast milk	10	18	Benzylpenicillin and cefotaxime
4	26	900	5	38–67	Expressed breast milk	23	13	Benzylpenicillin, cefotaxime and flucloxacillin, gentamicin, fucidin, and amikacin
5	28	1120	8	26–71	Expressed breast milk	14	20	Benzylpenicillin and cefotaxime
6	28	1220	1	37	Formula feed (SMA)	27	15	Benzylpenicillin, cefotaxime, flucloxacillin, and gentamicin
7	28	1160	1	54	Low birthweight formula feed (SMA)	40	34	Benzylpenicillin and cefotaxime
8	28	1160	1	21	Formula feed (SMA)	10	11	Benzylpenicillin and cefotaxime
9	29	1160	4	14–43	Total parenteral nutrition, plus expressed breast milk for 24 hours	1	0	Benzylpenicillin and cefotaxime; in progress: flucloxacillin and gentamic in gentamicin
10	30	1610	1	29	Expressed breast milk	10	4	Benzylpenicillin, cefotaxime, azlocillin, and gentamicin
11	31	1170*	2	29 and	Expressed breast milk	20	20	Benzylpenicillin and cefotaxime
				53	Expressed breast milk	44	44	Benzylpenicillin and cefotaxime
12	31	1860	2	15–22	Total parenteral nutrition and expressed breast milk	5	7	Benzylpenicillin and cefotaxime
13	34	1760*	1	21	Formula feed (Osterfeed)	21	21	None
14	34	2370	2	6–14	Formula feed (SMA) and dextrose	6	3	Benzylpenicillin and cefotaxime
15	34	1400*	2	10–12	Low birth weight formula feed (SMA)	10	10	None
16	34	2330	1	8	Formula feed (SMA)	8	5	Benzylpenicillin and cefotaxime
17	34	2410	2	15–22	Expressed breast milk and formula feed (SMA)	15	10	Benzylpenicillin and cefotaxime
18	34	1420*	2	23–29	Formula feed (SMA)	23	23	None
Methylmalonic acid								
4	26	900	4	38–67	Expressed breast milk	23	13	Benzylpenicillin; cefotaxime, flucloxacillin, gentamicin, fucidin, and amikacin
D-lactic acid								
2	26	940	1	28	Expressed breast milk	9	9	Benzylpenicillin, cefotaxime, flucloxacillin, gentamicin, fucidin, and amikacin
3	26	862	1	30	Expressed breast milk	10	18	Benzylpenicillin and cefotaxime
†4	26	900	1	38	Expressed breast milk	23	13	Benzylpenicillin, cefotaxime, flucloxacillin, gentamicin, and fucidin
5	28	1120	2	43–51	Expressed breast milk	29	35	Benzylpenicillin and cefotaxime
9	29	1160	1	22	Expressed breast milk	3	2	Benzylpenicillin, cefotaxime, flucloxacillin and gentamicin

*Birth weight less than 10th centile for gestation; †insufficient urine to confirm acid present.

nine). Significantly more VLBW babies (12 of 21, 57%) excreted the diol than babies born weighing more than 1500 g (six of 31, 19%, $p<0\cdot01$), and four of the five babies weighing less than 1000 g at birth were diol excretors. The fifth infant had necrotising enterocolitis and received parenteral nutrition and intravenous antibiotics throughout the monitoring period. Four of 13 (31%) babies born small for gestational age excreted the diol. This was similar to the incidence for babies of appropriate weight for gestational age (14 of 39, 36%; $p>0\cdot05$).

URINARY EXCRETION OF OTHER CARBOHYDRATE METABOLITES

Methylmalonic acid was detected in four samples collected from one baby (gestational age 26 weeks) between 38 and 67 days of life. On three occasions small amounts of ethylmalonic acid were also found. High concentrations of lactic acid (37, 56, and 102 mg/mmol creatinine), respectively were present in three urine samples from two babies born before 33 weeks who were not acidotic, and there were moderately increased concentrations in three others. 2,3-Butanediol was also present in four of these samples, and it was suspected that they might contain bacterially produced D-lactic acid in addition to endogenous L-lactic acid. These isomers are not separated by our normal chromatographic procedure. Five of the samples were analysed using an enzyme method,[14] and D-lactic acid was confirmed. This acid may also have been present at lower concentration in other samples, but it was not feasible to reanalyse all the specimens.

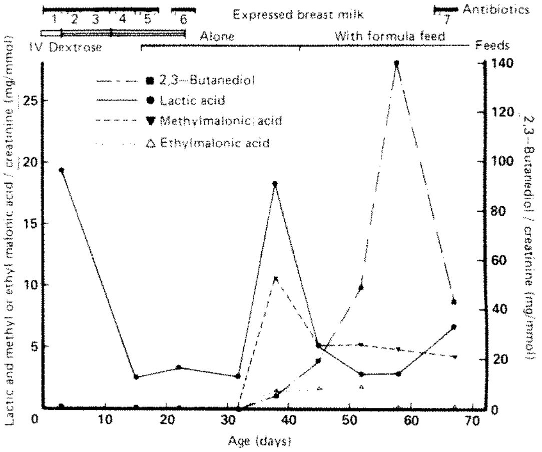

Fig 2 *Excretion of bacterial carbohydrate metabolites by case 4, born at 26 weeks' gestation, birth weight 900 g. She required artificial ventilation for 31 days. Necrotising entercolitis developed at 73 days. Intravenous antibiotic treatment—1: Benzylpenicillin and cefotaxime. 2: Benzylpenicillin, flucloxacillin, cefotaxime, and gentamicin. 3: Fucidin, amikacin and flucloxacillin. 4: Flucloxacillin and cefotaxime. 5: Benzylpenicillin and cefotaxime. 6: Flucloxacillin and cefotaxime. 7: Netilmicin and cefotaxime.*

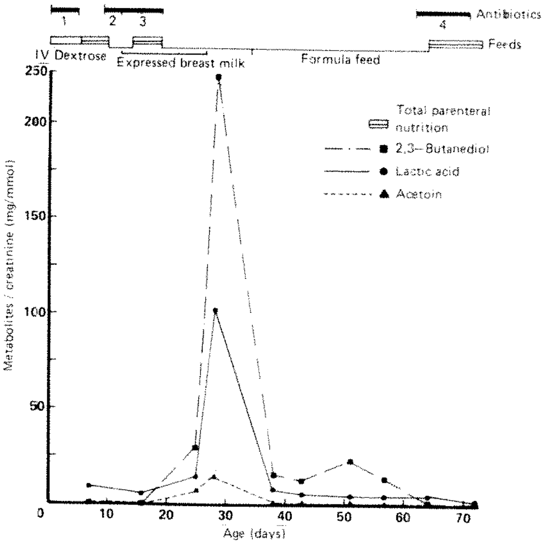

Fig 1 *Excretion of bacterial carbohydrate metabolites by case 2, born at 26 weeks' gestation, birth weight 940 g. She required artificial ventilation for 28 days. Necrotising enterocolitis developed at 60 days. Intravenous antibiotic treatment—1: Benzylpenicillin and cefotaxime. 2: Flucloxacillin, gentamicin, and fucidin. 3: Amikacin and fucidin. 4: Flagyl, ampicillin, and gentamicin.*

EXCRETION OF METABOLITES RELATED TO FEEDING, ANTIBIOTIC TREATMENT, AND MICROBIOLOGY OF RECTAL SWABS

Excretion of the abnormal carbohydrate metabolites was not a constant feature; only one episode coincided with the onset of proved or suspected necrotising enterocolitis, and this was in a baby who had been excreting 2,3-butanediol for at least 14 days. Table 3 summarises preceding antibiotic treatment, and the type of feeding being given when the urinary abnormality was first noticed. All the babies who excreted the metabolites were being fed orally. The products were not present in any of the 16 samples collected from 14 babies during total parenteral nutrition. Fourteen of these 16 collections were during courses of antibiotic treatment. In most cases, feeds were well established (five to 40 days). Two babies receiving parenteral nutrition, however,

Table 4 *Coliform micro-organisms grown from rectal swabs from three babies who excreted 2,3-butanediol*

Case No	*Gestation (weeks)*	*Chronological age (days)*	*Urinary 2,3-butanediol (mg/mmol creatinine)*	*Faecal bacteria*	*Sensitive to cefotaxime*
2	26	28	224·0	*Enterobacter agglomerans*	Yes
		38	15·9	*Klebsiella pneumoniae*	Yes
				Escherichia coli	Yes
		43	12·8	*Escherichia coli*	Yes
		57	13·5	*Klebsiella oxytoca*	Yes
				Escherichia coli	Yes
4	26	45	18·9	*Enterobacter cloacae*	No
		52	50·2	*Serratia marcescens*	Yes
		58	139·3	*Klebsiella pneumoniae*	Yes
5	28	43	136·1	*Klebsiella pneumoniae*	Yes
				Enterobacter cloacae	Yes
		51	11·3	*Klebsiella pneumoniae*	Yes

had started oral feeds with small amounts of expressed breast milk (1 ml/hour) within the preceding 24 hours. Some babies were being fed with formula feeds when the metabolites were found, but most immature babies were receiving expressed breast milk. Excretion of detectable amounts of 2,3-butanediol by three babies ceased when oral feeds were discontinued and parenteral nutrition and treatment with broad spectrum antibiotics was instituted.

Generally the urinary abnormality was first observed some days after discontinuing antibiotic treatment (three to 34 days, median 12, n=16) (figs 1 and 2). Two babies receiving parenteral nutrition, however, were receiving gentamicin and flucloxacillin.

Fifteen rectal swabs were taken for microbiological studies from seven babies within 48 hours of collecting urine for organic acid studies. Three of the babies excreted 2,3-butanediol on nine occasions (table 4). On eight of these enterobacter, klebsiella, or serratia were identified in faecal cultures. *Enterobacter cloacae* was also cultured from two of six samples collected from the four remaining babies, who did *not* excrete the diol, but klebsiella and serratia were not isolated. Yeast organisms were grown from the groins of one baby at the time of study and there was perianal soreness, but no rectal swabs were examined in this case. No other baby was recorded as being colonised with yeast or fungi at the time of excretion of 2,3-butanediol.

Discussion

In this study, metabolites of carbohydrate fermentation produced by gut micro-organisms were found in the urine of preterm babies in a neonatal unit. The urinary abnormalities occurred particularly among VLBW babies born before 33 weeks' gestation. They excreted 2,3-butanediol more often and in higher concentrations than babies of 33 to 36 weeks' gestation. Acetoin, methylmalonic acid, and appreciable amounts of D-lactic acid were detected only in this group. The abnormalities occurred as transient episodes, always after introducing oral feeding. In three cases excretion ceased when oral feeds were withdrawn and antibiotic treatment started.

Facultative anaerobes (enterobacteriaceae) were almost certainly the sources of 2,3-butanediol and acetoin.[1 15] These can be subdivided into organisms that produce these compounds as their main carbohydrate fermentation products (species of the genera klebsiella, enterobacter, and serratia) and those that do not (for example *Escherichia coli*). Acetoin is an intermediate in the fermentation pathway and is the metabolic precursor of 2,3-butanediol.[13] On eight of nine occasions when three babies excreted 2,3-butanediol, bacteria of the species klebsiella, enterobacter, or serratia were cultured from rectal swabs and may be assumed to have been the source of the urinary metabolite. The case for *E cloacae* is weakened by the observation that this organism was also grown from swabs from two babies who did not excrete the diol. Small numbers of these organisms in the gut, colonic conditions unfavourable for diol production (for example, pH above 6·0[1]), or decreased intestinal absorption of the metabolite, however, might all be offered as explanations for the absence of the diol in these two babies. Yeasts also produce acetoin,[13] and may have accounted for 2,3-butanediol excretion in one baby, although we do not know definitely whether she had simultaneous gut and skin colonisation with these organisms.

Lactobacilli may have been the source of D-lactic acid as in older subjects following bowel resection.[8 9] Other organisms, however, including *E coli*, pseudomonas, and *Klebsiella pneumoniae*, sometimes produce D-lactic acid as metabolic intermediate, and might have been responsible.[1] Methylmalonic acid was excreted by one baby who did not have an inborn error of metabolism, and in whom vitamin B_{12} deficiency was an unlikely explanation in view of earlier intravenous supplementation with 0·2 μg per kg of vitamin B_{12} daily. Methylmalonic acid is produced by anaerobes such as the bacteroidaceae (including *Bacteroides fragilis*) as an intermediate in glucose fermentation.[1, 15] Butyric acid—an end product of the pathway—may have been the precursor of ethylmalonic acid, which was also detected in this baby's urine.

To our knowledge acetoin has not been found in urine before, and the other carbohydrate fermentation products found have been encountered only rarely in older subjects. What is it that makes premature babies unique? Firstly, they have increased intestinal permeability compared with babies born at full term, and the bacterial metabolites were probably well absorbed. This is particularly likely among the immature babies, and probably helps to explain why they excreted the metabolites more often than babies born after 33 weeks' gestation. The key feature, however, is probably that preterm infants have markedly impaired intestinal absorption of lactose.[5, 6] This reflects the late development of intestinal lactase which, at 26 to 34 weeks' gestation, is only 30% of the activity at full term.[16] The colonic bacteria are therefore exposed to large amounts of unabsorbed sugar that they can use as metabolic substrate. This is in some ways analogous to older subjects after ileal resection. The ready supply of carbohydrate may favour growth of enterobacteriaceae and would thereby underly another important feature of prematurity—namely, the high ratio of facultative anaerobes to strict anaerobes among the colonic microflora compared with babies born at full term,[17] which persists for several weeks.[18] Colonisation with enterobacter and klebsiella may occur often among babies on neonatal intensive care units,[19] and is favoured by antibiotic regimens in common use. Overgrowth with klebsiella was reported during treatment with five different combinations of systemically administered antibiotics,[17] and high counts of klebsiella and enterobacter reported after treatment.[17, 20] Development of antibiotic resistance, common among these bacteria,[21] would be a further factor in promoting their growth. Most babies in this study had received broad spectrum antibiotics before the appearance of 2,3-butanediol in their urine.

The findings support evidence from breath hydrogen analysis of bacterial carbohydrate fermentation in the gut of preterm infants.[5] By identifying a biochemical marker for enterobacter, klebsiella, and serratia, it has been possible to recognise episodes in which colonisation with these opportunistic, potential pathogens,[22] probably occurred. Carefully combined microbiological and biochemical studies may increase our understanding of the peculiarities of the gut ecology in preterm babies, which must be a factor in their predisposition to develop necrotising enterocolitis. It has been proposed that administration of lactobacilli may be beneficial for certain newborn infants.[23] The possible production of carbohydrate fermentation products that might have toxic effects[8, 9] as a result of such intervention requires more detailed investigation.

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References

1 Gottschalk G. *Bacterial metabolism*. 2nd ed. New York: Springer-Verlag, 1986.
2 McNeil NI, Cummings JH, James WPT. Short chain fatty acid absorption by the human large intestine. *Gut* 1978;**19**:819–22.
3 Bain MD, Borriello SP, Tracey BM, *et al.* Contribution of gut bacterial metabolism to human metabolic disease. *Lancet* 1988;**i**:1078–9.
4 Walter JH, Leonard JV, Thompson GN, Halliday D, Bartlett K. Gut bacterial metabolism. *Lancet* 1988;**ii**:226.
5 MacLean WC, Fink BB. Lactose malabsorption by premature infants: magnitude and clinical significance. *J Pediatr* 1980;**97**:383–8.
6 Johnson JD. Antibiotics and carbohydrate malabsorption in newborns. *Am J Dis Child* 1986;**140**:101–2.
7 Garcia J, Smith FR, Cucinell SA. Urinary D-lactate excretion in infants with necrotizing enterocolitis. *J Pediatr* 1984;**104**:268–70.
8 Oh MS, Phelps KR, Traube M, *et al.* D-lactic acidosis in a man with the short-bowel syndrome. *N Engl J Med* 1979;**301**:249–52.
9 Haan E, Brown G, Bankier A, *et al.* Severe illness caused by the products of bacterial metabolism in a child with a short gut. *Eur J Pediatr* 1985;**144**:63–5.
10 Flannery D, Lafer CZ, Roesel RA. Gut bacterial metabolism. *Lancet* 1988;**ii**:225–6.
11 Hommes FA, Bruins AP, Dajani-Wielaard J, Baarsma R. 2,3-Dihydroxybutane: an unusual compound found in the gas chromatographic analysis of volatile compounds of urine. *J Clin Chem Clin Biochem* 1979;**17**:97–9.
12 Francis D. *Nutrition for children*. Oxford: Blackwell, 1986:30–1.
13 Mills GA, Walker V. Urinary excretion of 2,3-butanediol and acetoin by babies on a special care unit. *Clin Chim Acta* 1989;**179**:51–60.
14 Gawhen K, Bergmeyer HU. D-(-)-lactate. In: Bergmeyer HU, ed. *Methods of enzymatic analysis*. Vol 3. New York: Academic Press, 1974:1492–3.
15 MacFaddin JF. *Biochemical tests for identification of medical bacteria*. 2nd ed. Baltimore: Williams and Wilkins, 1980.
16 Antonowicz I, Lebenthal E. Developmental pattern of small intestinal enterokinase and disaccharidase activities in the human fetus. *Gastroenterology* 1977;**72**:1299–303.
17 Bennet R, Eriksson M, Nord CE, Zetterstrom R. Fecal bacterial microflora of newborn infants during intensive care management and treatment with five antibiotic regimens. *Pediatr Infect Dis* 1986;**5**:533–9.
18 Sakata H, Yoshioka H, Fujita K. Development of the intestinal flora in very low birth weight infants compared to normal full-term newborns. *Eur J Pediatr* 1985;**144**:186–90.

[19] Goldmann DA, Leclair J, Macone A. Bacterial colonization of neonates admitted to an intensive care environment. *J Pediatr* 1978;**93**:288–93.

[20] Bennet R, Nord CE. Development of the faecal anaerobic microflora after caesarean section and treatment with antibiotics in newborn infants. *Infection* 1987;**15**:332–6.

[21] Borderon JC, Gold F, Laugier J. Enterobacteria of the neonate. *Biol Neonate* 1981;**39**:1–7.

[22] Clark DA, Thompson JE, Weiner LB, *et al.* Necrotizing enterocolitis: intraluminal biochemistry in human neonates and a rabbit model. *Pediatr Res* 1985;**19**:919–21.

[23] Goldmann DA. The bacterial flora of neonates in intensive care—monitoring and manipulation. *J Hosp Infect* 1981;**11** (suppl A):340–51.

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Left ventricular output during postnatal circulatory adaptation in healthy infants born at full term

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SUMMARY Left ventricular output was measured non-invasively at predefined time intervals from <15 minutes to 72 hours after birth in 16 infants who had been born at full term. The blood flow velocity in the ascending aorta was measured by a range gated Doppler technique and multiplied by the cross sectional diameter measured by cross sectional and M mode echocardiography. Left ventricular output remained high in the first two hours, 235–243 ml/min/kg, despite a 10% decrease in heart rate. The fall in heart rate was compensated for by a 15% increase in stroke volume. Between 2 and 24 hours there was a significant fall in mean (SD) left ventricular output to 187 (35) ml/min/kg caused mainly by a reduction in stroke volume. The fall in left ventricle output after two hours may reflect an adaptation to the decreased demand on the left ventricle as the ductus constricts.

Postnatal circulatory transition entails fundamental changes in the systemic and pulmonary circulations.[1] During fetal life the two ventricles work in parallel, the left ventricle mainly supplying the upper body with blood, particularly the myocardium and the brain. After birth and completed circulatory transition, the left ventricular output provides all systemic perfusion. This leads to an increased volume demand on the left ventricle.[1] The physiological left to right shunt through the open ductus arteriosus soon after birth may also contribute to an increased volume load on the left ventricle.

The ability to increase output by increased stroke volume is thought to be limited in the fetus and newborn because of lower myocardial compliance[2] and contractile reserve[3] compared with the adult. Despite this, left ventricular output in the lamb is more than doubled after birth.[4] Because non-invasive methods of investigation have been developed relatively recently, most of our knowledge about the haemodynamic changes after birth is based on studies in animals. With ultrasound imaging and Doppler techniques left ventricular output can now be studied non-invasively in newborn infants as well. Several non-invasive studies have been carried out in the postnatal period but in no study has left ventricular output been measured directly after birth.[5-7]

The aim of this investigation was to study the postnatal changes in the left ventricular output of newborn infants by frequent measurements at precise time intervals starting almost immediately after birth.

Subjects and methods

Sixteen healthy infants born at full term, each with a normal neonatal course, were studied. Infants born by elective caesarean sections were chosen so that the first recording could be made within 15 minutes of the birth. Indications for caesarean section were: cephalopelvic disproportion, breech presentation, and previous caesarean section. Maternal epidural anaesthesia (bupivacaine) was used in 13 cases, and general anaesthesia (barbiturates, halothane, and nitrous oxide) in three cases.

Blood samples were taken at birth from the umbilical arteries of the double clamped cord for measurement of pH and packed cell volume. A combined range gated and continuous multifrequency Doppler velocimeter (ALFRED, Vingmed A/S) was used to measure blood flow velocities in the ascending aorta. A high pass filter was set at 400 Hz to reduce noise from vessel wall motions. With the infant supine under a radiant warmer, the 5 MHz transducer with a tip 8 mm in diameter was positioned in the suprasternal notch. Guided by the audio signal, the transducer was directed towards the ascending aorta. The echoes from the aortic valves were identified and the sample volume, with a length of 4 mm, was placed 5–10 mm above the valves. Care was taken to find the most high pitched frequencies by small changes in the probe angle. The space average velocity (the average over the cross section of the aorta) (fig 1) and the temporal mean of the space average velocity signals were recorded on an oscillograph (Mingograph 34, Siemens Elema). A stable recording over 20–30 heart beats with the highest Doppler shift was selected, and the corresponding mean flow velocity was determined.

Cross sectional and M mode echocardiography were performed immediately after each Doppler recording using a 7·5 MHz sector scanner and an echocardiograph (Technicare). The end systolic internal diameter (trailing edge to leading edge) of the ascending aorta, 5–10 mm above the valves, was measured on a hard copy of the parasternal long axis M mode recording, and a mean taken over 8–10 consecutive heart beats (fig 2). The calculated cross sectional area was multiplied by the mean flow velocity, and divided by the body weight to give left ventricular output in ml/min/kg. Stroke volume was calculated by dividing left ventricular output by heart rate. Blood pressure was measured non-invasively in connection with each Doppler recording using the oscillometric technique (Omega 1400, In Vivo Research Laboratories). The width of the cuff on the right upper arm was carefully chosen to minimise errors.[8]

All recordings on the same infant were made by the same investigator, and were performed at <15 minutes, 30 minutes, 2 hours, 5 hours (4–6), 24 hours (22–26), and 72 hours (70–74) after birth. The two last recordings were performed about 30 minutes after feeding. The sleep stage was clinically assessed as quiet sleep or rapid eye movement sleep. Informed consent was obtained from the parents before delivery, and the study was approved by the local committee of ethics. The statistical evaluation was made by multifactorial analysis of variance, using a model where individual subjects, postnatal age, and sleep stage were sources of variation. Tukey's hourly significant difference test was used for multiple comparisons of means.[9]

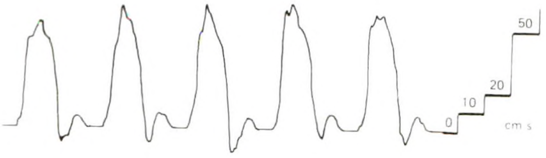

Fig 1 *Average cross sectional flow velocity recording from ascending aorta.*

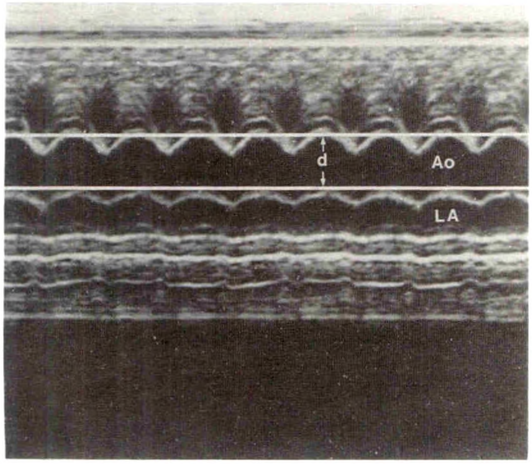

Fig 2 *Long axis M mode recording from ascending aorta (Ao), and left atrium (LA). Measurement of internal aortic diameter (d) in end systole.*

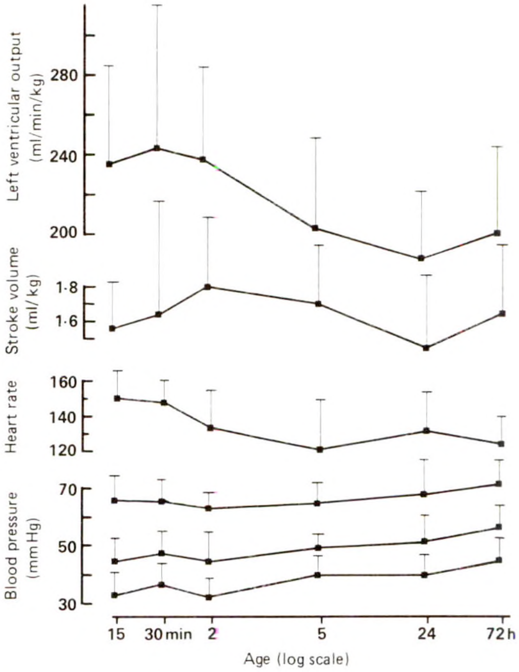

Fig 3 *Mean (SD) left ventricular output, stroke volume, heart rate, and arterial blood pressure during postnatal circulatory adaptation in 16 healthy infants born at full term.*

Results

Each infant was healthy and had a normal neonatal course. Mean (SD) gestational age was 38·5 (0·7) weeks and birth weight was 3370 (344) g. Umbilical arterial blood pH and packed cell volume were 7·32 (0·04), and 0·46 (0·05), respectively. Recordings were obtained on all occasions in all infants. The Doppler measurements were made at a sampling depth of 3·3 (0·3) cm.

Mean left ventricular output, stroke volume, heart rate, and arterial blood pressure are shown in fig 3. Heart rate decreased with increasing age and was significantly lower at 2 hours than at birth ($p<0·01$). Blood pressure did not change significantly before 2 hours. Between 2 and 72 hours systolic, diastolic, and mean pressure rose significantly ($p<0·01$). Pulse pressure decreased from 31 (4·8) to 25 (6·9) mm Hg between 2 and 5 hours ($p<0·01$). The mean diameter of the ascending aorta was 9·8 (1·0) mm. No significant differences in aortic diameter were obtained between measurements. The variation coefficients for the aortic diameter measurements varied among the infants from 2 to 13% (mean 6%).

Left ventricular output remained high for the first two hours, decreased at five hours in 13 of the 16 infants ($p<0·01$), and then decreased further until 24 hours in 11/16 infants ($p<0·01$). Left ventricular output was lower ($p<0·01$) when the infants were sleeping but did not differ between quiet and rapid eye movement sleep. Left ventricular stroke volume increased from the first measurement to a maximum at 2 hours ($p<0·01$), and then decreased until 24 hours ($p<0·01$).

Discussion

In this study left ventricular output was determined at several predefined time intervals after birth. The recording intervals were chosen so that the main circulatory changes during the normal transition should be included. Using strictly non-invasive techniques, minimal stress was inflicted on the infants and the transition itself should accordingly not have been disturbed. Only infants born by caesarean section were included. The lower plasma catecholamine concentrations found in infants born this way[10] and the drugs used for maternal anaesthesia might influence both myocardial contractility[11] and left ventricular output.[12] The smaller placental transfusion during caesarean section could also affect the cardiovascular state.[13] Differences in left ventricular output have also been reported. Gessner *et al* showed somewhat higher left ventricular output in vaginally delivered infants compared with those delivered by caesarean section, but they found no difference in ductus shunting during the first few hours after birth.[14]

A close correlation has been found between invasive methods and the Doppler technique for measurement of cardiac output in newborn and older infants, although the Doppler does not include coronary blood flow.[5 15 16] We calculated left ventricular output from the mean flow velocity and the inner diameter of the ascending aorta. Others have used the leading edge method (from the external surface of the anterior aortic wall to the internal surface of the posterior wall)[6 15] as proposed by the American Society of Echocardiography in 1978,[17] as these structures may be easier to define and should permit more precise measurements in adults. The leading edge method overestimates the diameter, and the left ventricular output, however, by including one vessel wall thickness. With the higher ultrasound frequencies used in neonates, and with modern ultrasound equipment, the trailing to leading edge method may be more appropriate. In spite of this, the mean diameter in our group was almost the same as that given by Walther *et al* in a similar group of infants, using the leading edge method.[18] The variations in aortic diameter between measurements probably reflect errors in method rather than true changes in diameter because there were no systematic changes over the observation period in our study. Accordingly, the mean diameter of each child was used for calculation of the left ventricular output. Goldberg *et al* did not overestimate left ventricular output with the leading edge method,[5] compared with dye dilution measurements, which is in contrast to the results of Mellander *et al*, who compared thermodilution with different ultrasound methods for measurement of left ventricular output.[16] Mellander *et al* reported that the leading edge method overestimated left ventricular output by 30%, compared with 2% for the inner diameter technique, and gave a somewhat higher variation. As the velocity profile in the ascending aorta is not flat,[19 20] using the maximum flow velocity gives an overestimation of flow whereas the mean flow velocity produces more correct results.[21] Although the maximum velocity recording is less dependent on complete insonation of the vessel, using it in neonates and older infants gives only a slightly smaller variation, while it produces a large overestimation of left ventricular output.[16] Hence the product of cross sectional mean flow velocity and the cross sectional area derived from the inner diameter should yield the best measure of the output.

The decrease in heart rate between the first measurement and 2 hours may reflect hormonal and metabolic changes—for example, the end of the catecholamine surge at birth.[22] Despite this, left ventricular output remained high. A possible explanation for the sustained high left ventricular output at 2 hours is that it is a consequence of a left to right shunt through the open ductus, which other studies have suggested occurs at this time.[14 23 24] This explanation is supported by the decrease in diastolic blood pressure, and increase in stroke volume up to 2 hours. From 2 to 5 hours and then to 24 hours, left ventricular output, stroke volume, and pulse pressure decreased. This favours the concept of a diminishing shunt starting before 5 hours of age. This seems to occur earlier than expected, but previous studies with invasive techniques may have interfered with the normal circulatory transition and delayed ductus constriction.[23 24] Echocardiographic signs of ductus constriction have also been shown recently at 4 hours in 80% of healthy infants born at full term.[25] Even though ductal shunting can be detected non-invasively with the Doppler technique 24 hours after birth in over half of the healthy infants born at full term,[26] this only gives a qualitative measure of the shunt and the time period when it is haemodynamically important may be much shorter and occur earlier. At present there is no available non-invasive technique for accurate quantitation of the ductus flow, but studies of left ventricular systolic time intervals also seem to show early disappearance of a haemodynamically important ductus shunt.[27]

The sleep/wake state may also influence left ventricular output. At the first two recordings the infants were awake and left ventricular output was high, whereas at the two last recordings most infants were asleep and left ventricular output was lower. Multifactorial analysis of variance, however, showed that postnatal age had a significant influence on left ventricular output independent of sleep/wake state.

The increase in stroke volume compensated for the fall in heart rate between 30 minutes and 2 hours, and left ventricular output was unchanged. Between 2 and 24 hours the stroke volume decreased, heart rate remained nearly constant, and left ventricular output declined. Thus left ventricular output seems to depend more on stroke volume than on heart rate during this period.

In this study left ventricular output was 237 ml/min/kg at 2 hours and 200 ml/min/kg after 24 hours. These values are of the same magnitude as has previously been reported in invasive studies. Gessner *et al* found left ventricular output to be 233 ml/min/kg between 30 minutes and 2 hours of age in infants born by caesarean section,[14] and Emmanouilides *et al* reported 209 ml/min/kg in newborn infants without shunts.[28] Previous Doppler studies have shown somewhat higher left ventricular output,[5 6] probably explained by differences in signal processing and measurements of the aortic diameter. If the systemic perfusion (ductus flow excluded) was maintained at 200 ml/min/kg, the measured left ventricular output suggests a 15–20% left to right shunt at 2 hours. If the shunt was larger, however, it would mean reduced systemic flow at this age. Whether the systemic perfusion is maintained or compromised by the ductus shunt cannot be determined from our data. The cerebral blood flow, however, does not seem to be reduced when assessed in a similar group of infants at the same time intervals.[29]

We conclude that left ventricular output remains high during early postnatal transition in spite of decreasing heart rate. A fall in left ventricular output occurs after 2 hours of age, possibly as a result of decreased demand on the left ventricle as the ductus constricts.

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References

[1] Rudolph AM. Changes in the circulation after birth. *Congenital heart diseases*. Chicago: Year Bood Medical Publishers, 1974: 17–28.

[2] Friedman WF. The intrinsic physiologic properties of the developing heart. *Prog Cardiovasc Dis* 1972;**15:**87–111.

[3] Friedman WF, Kirkpatrick SE. In situ physiological study of the developing heart. *Recent Advances in the Study of Cardiac Structure and Metabolism* 1975;**5:**497–504.

[4] Klopfenstein HS, Rudolph AM. Postnatal changes in the circulation, and responses to volume loading in sheep. *Circ Res* 1978;**42:**839–45.

[5] Alverson DC, Eldridge MW, Dillon T, Yabek SM, Berman W. Noninvasive pulsed Doppler determination of cardiac output in neonates and children. *J Pediatr* 1982;**101:**46–50.

[6] Walther FJ, Siassi B, Ramadan NA, Ananda A, Wu PYK. Pulsed Doppler determinations of cardiac output in neonates: normal standards for clinical use. *Pediatrics* 1985;**76:**829–33.

[7] Hirsimäki H, Kero P, Wanne O, Erkola R, Mako Z. Doppler derived cardiac output in healthy newborn infants in relation to physiological patency of the ductus arteriosus. *Pediatr Cardiol* 1988;**9:**79–83.

[8] Sonesson S-E, Broberger U. Arterial blood pressure in the very low birth weight neonate. *Acta Paediatr Scand* 1987;**76:**338–41.

[9] Daniel W. *Biostatistics: a foundation for analysis in the health sciences*. 3rd ed. New York: John Wiley, 1983:224–6.

[10] Irestedt L, Lagercrantz H, Hjelmdahl P, Hägnevik K, Belfrage P. Fetal and maternal plasma catecholamine levels at elective cesarean section under general or epidural anesthesia versus vaginal delivery. *Am J Obstet Gynecol* 1982;**142:**1004–10.

[11] Block A, Covino BG. The effect of local anesthetic agents on cardiac conduction and contractility. *Reg Anaesth* 1981;**6:**55–61.

[12] Liu P, Feldman HS, Covino BM, Giasi BR, Covino BG. Acute cardiovascular toxicity of intravenous amide local anesthetic in anesthetized ventilated dogs. *Anesth Analg* 1982;**61:**317–22.

[13] Yao AC, Lind J. *Placental transfusion*. Springfield: Charles C Thomas, 1982:103–7.

[14] Gessner I, Krovetz LJ, Bensin RW, Prystowsky H, Stenger V, Eitzman DV. Hemodynamic adaption in the newborn infant. *Pediatrics* 1965;**36:**752–62.

[15] Goldberg S, Sahn D, Allen H, Valdes-Cruz L, Hoenecke H, Carnahan Y. Evaluation of pulmonary and systemic blood flow by 2-dimensional Doppler echocardiography using fast Fourier transform spectral analysis. *Am J Cardiol* 1982;**50:**1394–400.

[16] Mellander M, Sabel K-G, Caidahl K, Solymar L, Eriksson B. Doppler determination of cardiac output in infants and children: comparison with simultaneous thermodilution. *Pediatr Cardiol* 1987;**8:**241–6.

[17] Sahn DJ, DeMaria A, Kissolo J, Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. *Circulation* 1978; **58:**1072–83.

[18] Walther FJ, Siassi B, King J, Wu PYK. Normal values of aortic root measurement in neonates. *Pediatr Cardiol* 1985;**6:**61–4.

[19] Clark C, Schultz DL. Velocity distribution in aortic flow. *Cardiovasc Res* 1973;**7:**601–13.

[20] Levy b, Targett RC, Bardou A, McIlroy MB. Quantitative ascending aortic Doppler blood velocity in normal human subjects. *Cardiovasc Res* 1985;**19:**383–93.

[21] Lundell BPW, Lindström DP, Arnold TG. Neonatal cerebral blood flow velocity. An in vitro validation of the pulsed Doppler technique. *Acta Paediatr Scand* 1984;**73:**810–5.

[22] Lagercrantz H, Bistoletti P, Nylund L. Sympathoadrenal activity in the fetus during delivery and at birth. In: Stern L, Salle B, Friis-Hansen B, eds. *Intensive care in the newborn*. New York: Masson, 1981:1–12.

[23] Adams FH, Lind J. Physiologic studies on the cardiovascular status of normal newborn infants (with special reference to the ductus arteriosus). *Pediatrics* 1957;**19:**431–7.

[24] Moss AJ, Emmanouilides G, Duffie ER Jr. Closure of the ductus arteriosus in the newborn infant. *Pediatrics* 1963;**30:** 25–30.

[25] Hiraishi S, Misawa H, Oguchi K, *et al.* Two-dimensional Doppler echocardiographic assessment of closure of the ductus arteriosus in normal newborn infants. *J Pediatr* 1987;**111:** 755–60.

[26] Gentile R, Stevenson G, Dooley T, Kawabori I, Pearlman A. Pulsed Doppler echocardiographic determination of time of ductal closure in normal newborn infants. *J Pediatr* 1981;**98:** 443–8.

[27] Lundell BPW, Wallgren CG. Assessment of left ventricular adaptation to extrauterine circulation. Systolic time intervals in the newborn infant. *Acta Paediatr Scand* 1982;**71:**745–52.

[28] Emmanouilides GC, Moss AJ, Monset-Couchard M, Marcano BA, Rzeznic B. Cardiac output in newborn infants. *Biol Neonate* 1970;**15:**186–97.

[29] Sonesson S-E, Winberg P, Lundell BPW. Early postnatal changes in intracranial arterial blood flow velocities in term infants. *Pediatr Res* 1987;**22:**461–4.

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Chorioamnionitis and serum IgM in Wilson-Mikity syndrome

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SUMMARY A total of 753 infants weighing less than 1800 g at birth were studied prospectively and their serum IgM concentrations measured within 72 hours of age. Placentas from 584 of these infants were examined histologically for chorioamnionitis. The results were correlated with chronic respiratory insufficiency. Altogether 101 infants developed chronic respiratory insufficiency of which 22 had bronchopulmonary dysplasia and 35 Wilson-Mikity syndrome. The remaining 44 infants were classified as 'unexplained chronic lung disease'. Mean serum IgM concentration for Wilson-Mikity syndrome was 1·02 g/l whereas it was 0·14 g/l for bronchopulmonary dysplasia and 0·32 g/l for unexplained chronic lung disease. The incidence of chorioamnionitis was significantly higher in Wilson-Mikity syndrome (30/35) compared with bronchopulmonary dysplasia (4/16) and with infants without chronic respiratory insufficiency (145/490). Wilson-Mikity syndrome was shown to be significantly correlated with the evidences of intrauterine inflammation.

Wilson-Mikity syndrome is a well documented form of chronic respiratory insufficiency of the newborn; the aetiology still remains unsettled. Extensive bacteriological and virological investigations have failed to show a common pathogenic organism.[1 2] Burnard *et al* considered this syndrome to lie at one end of a spectrum of deranged physiology caused by the mechanical disadvantages in the thorax and airways of immature babies.[3]

Pathology findings were detailed by Hodgman *et al.*[4] They performed 10 biopsies and 12 postmortem examinations, and of these 22 cases 12 were studied within 60 days of age. They found areas of hyperinflation and collapse in the first stage, followed by diffuse overinflation during the second stage. Pulmonary fibrosis was found in two cases. No evidence of infection or inflammation was reported.

Our preliminary observations showed that in most infants with Wilson-Mikity syndrome serum IgM measured within 4 days of age was significantly raised.[5] In the study reported here we investigated the evidence for intrauterine infection preceding Wilson-Mikity syndrome by studying infants with other forms of chronic respiratory insufficiency as controls.

Patients and methods

A prospective study was undertaken of infants admitted to our unit between 1982 and 1986. During this period 1611 infants were admitted of whom 657 infants were ventilated. The overall mortality was 162 (10·1%). There were 181 infants with respiratory distress syndrome of whom 33 (18·2%) infants died.

Altogether 753 infants, who weighed less than 1800 g and who survived for more than six days, were enrolled into the study. We excluded those infants whose neonatal serum samples were not available or who failed follow up to six months. Infants with chronic respiratory difficulty due to upper airway abnormality or poor respiratory drive were also excluded. There were 594 inborn and 159 outborn infants; 370 infants with respiratory distress syndrome or with other respiratory difficulties were ventilated according to the accepted guidelines.[6] The diagnosis of respiratory distress syndrome was based on clinical signs, chest radiography, and on the assessment of surfactant in gastric or tracheal aspirate using the lecithin:sphingomyelin ratio or microbubble test.[7] None of the infants with respiratory distress syndrome was the subject of artificial surfactant treatment.

Chronic respiratory insufficiency was defined as: prolonged intercostal retractions and a requirement for supplemental oxygen for more than four weeks.[8] The chest radiographs were interpreted by radiologists without knowledge of the IgM results.

Infants with chronic respiratory insufficiency were subdivided into three groups. The diagnosis of Wilson-Mikity syndrome was made if the infants satisified the following conditions: absence of respiratory distress syndrome and a chest radiograph showing diffuse, streaky infiltrate with small cystic areas appearing within four weeks.[4] The criteria for the diagnosis of bronchopulmonary dysplasia was ventilation for respiratory distress syndrome during the early neonatal period and, between 14–35 days of age, a chest radiograph showing at least stage II bronchopulmonary dysplasia.[9 10] Other infants with chronic respiratory insufficiency for more than four weeks who did not fulfil the criteria for Wilson-Mikity syndrome or bronchopulmonary dysplasia were described as having 'unexplained chronic lung disease'. These babies did not suffer respiratory distress syndrome and did not have radiological changes consistent with Wilson-Mikity syndrome or bronchopulmonary dysplasia.

Cord blood or a blood sample obtained within 72 hours of birth was analysed for serum IgG, IgA, and IgM and measured by radial immunodiffusion (Behring) in duplicate.

The placenta and umbilical cord underwent histological examination: after noting the macroscopic appearance they were fixed in formalin and chorioamnionitis was assessed by polymorphonuclear leucocytic infiltration into the fetal surface of the placenta and graded I, II, or III according to the extent of leucocyte invasion of the chorionic membrane or amniotic membrane respectively.[11] The diagnosis of subacute necrotising funisitis was based on the macroscopic and microscopic deposition of necrotic cell debris in the Wharton's jelly around the umbilical vessels. Some samples were calcium positive as shown by the staining of Kossa. The significance of difference in group comparisons was assessed using the χ^2 test with Yates's correction and the Student's t test.

Results

Of the 753 infants studied, 101 (13·4%) developed chronic respiratory insufficiency. There were 35 infants with Wilson-Mikity syndrome, 22 with bronchopulmonary dysplasia, and 44 with unexplained

Table 1 *Characteristics of infants studied*

Group	*No of infants (No who died)*	*Male/Female*	*Mean (SD) gestation (weeks)*	*Mean (SD) birth weight (g)*	*Duration of intermittent positive pressure ventilation*		*No with respiratory distress syndrome*
					No of days	*Median (range)*	
Wilson-Mikity syndrome	35 (1)	15/20	28·9 (2·3)	1084 (261)	23	5 (0–320)	0
Bronchopulmonary dysplasia	22 (1)	14/8	27·9 (2·6)*	937 (315)*	22	13 (3– 74)	22
Unexplained chronic lung disease	44 (3)	29/15	27·4 (2·6)	917 (280)	37	29 (0– 90)	0
Others†	652 (18)	291/361	31·2 (2·9)	1336 (300)	288	3 (0–100)	109

*p<0·01.
†Infants weighing <1800 g, who survived for more than six days, and who did not have chronic respiratory insufficiency.

Table 2 *Serum IgM, IgG, and IgA concentrations within 72 hours of age (g/l)*

Group	*IgM**		*IgG*		*IgA*	
	Mean (SD)	*Range*	*Mean (SD)*	*Range*	*Mean (SD)*	*Range*
Wilson-Mikity syndrome	1·02–(1·04)	0·06–3·77	5·80–(2·00)	2·72–11·52	0·05 (0·09)	0–0·44
Bronchopulmonary dysplasia	0·14–(0·08)	0·05–0·31	5·52–(2·06)	2·41– 9·63	0·02 (0·05)	0–0·23
Unexplained chronic lung disease	0·32–(0·42)	0·05–1·79	4·83–(1·84)	1·72– 8·80	0·03 (0·08)	0–0·39
Others*	0·21–(0·35)	0·02–3·46	7·16–(2·73)	1·80–19·14	0·01 (0·05)	0–0·47

*Wilson-Mikity syndrome compared with bronchopulmonary dysplasia, unexplained chronic lung disease, and others: p<0·001.
†Infants weighing <1800 g, who survived for more than six days, and who did not have chronic respiratory insufficiency.

Table 3 *Association between chorioamnionitis, subacute necrotising funisitis, and chronic lung disease*

Group	*Absent*	*Chorioamnionitis*			*Subacute necrotising funisitis*	
		I	*II*	*III*	*Absent*	*Present*
Wilson-Mikity syndrome	5	0	6	24*	18	17†
Bronchopulmonary dysplasia	12	2	2	0	16	0
Unexplained chronic lung disease	10	4	7	22	42	1
Others‡	345	32	54	59	477	13

Values indicate number of cases. The sample number available for analysis was 584 among 753 infants studied.
*Wilson-Mikity syndrome compared with bronchopulmonary dysplasia and Wilson-Mikity syndrome compared with others: $p<0{\cdot}001$.
†Wilson-Mikity syndrome compared with other group: $p<0{\cdot}001$. ‡Infants weighing <1800 g, who survived for more than six days, and who did not have chronic respiratory insufficiency.

chronic lung disease. Their characteristics are shown in table 1, together with infants without chronic respiratory insufficiency. Infants with unexplained chronic lung disease were more preterm and of lower birth weight compared with infants with Wilson-Mikity syndrome.

The mean serum IgM concentration within 72 hours of age was significantly higher in the group with Wilson-Mikity syndrome compared with the other individual groups ($p<0{\cdot}001$) (table 2). No differences were found in serum IgG or in IgA between each group or in IgM between the groups without Wilson-Mikity syndrome.

The placenta and cord were available for analysis in 584 (78%) infants. They were examined for inflammatory changes. The group with Wilson-Mikity syndrome had a significantly higher incidence of chorioamnionitis than the group with bronchopulmonary dysplasia or those without chronic respiratory insufficiency ($p<0{\cdot}001$) (table 3).

Subacute necrotising funisitis has been shown to correlate with intrauterine infection and with chorioamnionitis.[12] In the subjects studied the incidence of subacute necrotising funisitis was significantly higher in the group with Wilson-Mikity syndrome ($p<0{\cdot}001$) (table 3).

Discussion

We believe this is the first study of the association between immunoglobulin concentrations, placental pathology, and chronic respiratory insufficiency.

Serum IgM has been shown to be within a limited range both for term and preterm neonates.[13] Its increase indicates intrauterine infection. In established intrauterine infection such as cytomegalovirus and rubella there is a rise of total serum IgM and specific IgM concentrations.

The association of chorioamnionitis and subacute necrotising funisitis with intrauterine infection has been well established, and is presumably due to ascending infection.[14] The increased incidence of chorioamnionitis and subacute necrotising funisitis in infants with Wilson-Mikity syndrome is further evidence of intrauterine infection.

Chorioamnionitis is common in preterm births but a high serum IgM does not always accompany chorioamnionitis. The incidence of chorioamnionitis correlates inversely with gestational age of birth, but IgM correlates neither with gestational age nor with chorioamnionitis.[15] The association between total IgM and chorioamnionitis is therefore not fully explained. The event which initiated the pathology in the fetus and the placenta for Wilson-Mikity syndrome might be a different one from that which causes the chorioamnionitis seen in unexplained chronic lung disease or in general preterm births.

The second point to be raised in this study is the difference in the incidence of chorioamnionitis between bronchopulmonary dysplasia and unexplained chronic lung disease. The original concept of bronchopulmonary dysplasia reported by Northway *et al* in 1967 was association with respiratory distress syndrome during the early neonatal period.[9] After the recognition of a group of infants who show similar chronic lung disease without respiratory distress syndrome the concept was expanded.[16] It is not known if there were any differences in the aetiology between those infants with and without respiratory distress syndrome. It is made clear in the present study that there is an important group of babies with chronic respiratory insufficiency who do not have typical radiological changes of Wilson-Mikity syndrome or bronchopulmonary dysplasia. Combined with the high incidence of chorioamnionitis in these infants, we speculate that the chorioamnionitis may be the predisposing factor in the aetiology of unexplained chronic lung disease. The possible mechanism of chorioamnionitis relating to chronic lung insufficiency is discussed below.

The incidence of Wilson-Mikity syndrome in western countries has been reported to be much less in recent years. This is not the case in Japan, and 4·8%–8·7% of infants born weighing less than 1500 g have been reported to have Wilson-Mikity syndrome.[17, 18] There are two possible explanations for this: the first is that the real incidence has become low in western societies, and the second could be that some cases of existing Wilson-Mikity syndrome are misdiagnosed and confused with other groups of chronic lung disease, particularly with bronchopulmonary dysplasia. It is not surprising that a disease of an ill defined nature like Wilson-Mikity syndrome can still go unrecognised.

Infants with Wilson-Mikity syndrome show an uneven distribution of air spaces throughout their lungs and establish typical emphysema in the first several weeks of life. The pathophysiological lesion of the syndrome lies in the terminal airways, as shown by several lung function studies.[19–21] Then what is the relevance of the high incidence of chorioamnionitis and increased serum IgM in these infants? In this context recent findings on neutrophil elastase in pulmonary emphysema may be important.[22, 23] As destruction of elastin and the concomitant loss of elastic recoil in the emphysematous lung have been well established, attention has been focused on elastase as the primary destructive agent in the pathogenesis of emphysema. We have shown that the fetus who later developed Wilson-Mikity syndrome was subjected to chorioamnionitis. It is reasonable to expect that in response to chorioamnionitis the neutrophils migrate into the airway down to the developing alveoli. The rise in serum IgM concentration may be the immunological response of the fetus to infection. In our preliminary observations we found an increase in the number of neutrophils in the tracheal aspirate of the newborns who later developed Wilson-Mikity syndrome.[24] The numbers of neutrophils in the amniotic fluid were also high. In the same infants high neutrophil elastase was found in the tracheal aspirate obtained in the first 12 hours of birth. The difference was significant compared with those of preterm infants without respiratory illness and with respiratory distress syndrome. We therefore speculate that, whatever the triggering factor may be, invasion of neutrophils is involved. The increased elastase overwhelms the fetal antiproteinase activity, then hydrolyses the elastin that maintains the patency of terminal airways. After the postnatal adaptation for extrauterine life, these infants then develop pulmonary emphysema a few days and weeks after birth.

It seems that chorioamnionitis alone is not enough to cause Wilson-Mikity syndrome. The raised serum IgM suggests that the fetus has made an immunological response. Infants with unexplained chronic lung disease showed little increase in serum IgM. It is therefore a possibility that this difference in IgM between Wilson-Mikity syndrome and unexplained chronic lung disease correlates with a variety of clinical pictures. It also remains to be clarified if the aetiology of chorioamnionitis is the same in Wilson-Mikity syndrome and unexplained chronic lung disease.

In conclusion, we have shown that Wilson-Mikity syndrome can be defined as a form of chronic respiratory insufficiency characterised by a raised neonatal serum IgM concentration and associated with chorioamnionitis. Wilson-Mikity syndrome should be clearly differentiated from bronchopulmonary dysplasia so as to contribute to the further understanding of chronic respiratory insufficiency of newborn infants.

References

[1] Baghdassarian OM, Avery ME, Neuhauser EBD. A form of pulmonary insufficiency in premature infants: pulmonary dysmaturity? *AJR* 1963;**89:**1020–31.

[2] Swyer PR, Delivoria-Papadopoulos M, Levison H, *et al.* The pulmonary syndrome of Wilson and Mikity. *Pediatrics* 1965;**36:** 374–84.

[3] Burnard ED, Grattan-Smith P, Picton-Warlow CG, Grauaug A. Pulmonary insufficiency in prematurity. *Aust Paediatr J* 1965;**1:** 12–38.

[4] Hodgman JE, Mikity VG, Tatter D, *et al.* Chronic respiratory distress in the premature infants: Wilson-Mikity syndrome. *Pediatrics* 1969;**44:**179–95.

[5] Fujimura M, Takeuchi T, Ando M, *et al.* Elevated immunoglobulin M levels in low birth-weight neonates with chronic respiratory insufficiency. *Early Hum Dev* 1983;**9:**27–32.

[6] Reynolds EOR. Ventilator therapy. In: Thibeault DW, Gregory GA, eds. *Neonatal pulmonary care.* Menlo Park, California: Addison-Wesley Publishing, 1979:217–36.

[7] Pattle RE. Maturity of fetal lungs tested by produciton of stable microbubbles in amniotic fluid. *Br J Obstet Gynaecol* 1979;**86:** 615.

[8] Toce SS, Farrell PM, Leavitt LA, *et al.* Clinical and roentgenographic scoring systems for assessing bronchopulmonary dysplasia. *Am J Dis Child* 1984;**138:**581–5.

[9] Northway WH, Rosan RC, Porter DY. Pulmonary disease following respiratory therapy of hyaline-membrane disease. Bronchopulmonary dysplasia. *N Engl J Med* 1967;**276:**357–68.

[10] Edwards D, Dyer WM, Northway WH. Twelve years' experience with bronchopulmonary dysplasia. *Pediatrics* 1977;**59:** 839–46.

[11] Blanc WA. Amniotic infection syndrome: pathogenesis, morphology and significance in circumnatal mortality. *Clin Obstet Gynecol* 1959;**2:**705.

[12] Navarro C, Blanc WA. Subacute necrotizing funisitis. A variant of cord inflammation with a high rate of perinatal infection. *J Pediatr* 1974;**85:**689–97.

[13] Berg T. Immunoglobulin levels in infants with low birth weights. *Acta Paediatr Scand* 1968;**57:**369–76.

[14] Benirschke K. Routes and types of infection in the fetus and the newborn. *Am J Dis Child* 1960;**99:**714–21.

[15] Fujimura M, Takeuchi T, Imai S, Nakayama M. Prolonged rupture of membranes correlated with perinatal variables. In: Maeda K, Okuyama K, Takeda Y, eds. *Recent advances in perinatology.* Amsterdam: Excerpta Medica, 1986:413–23.

[16] Samuels MP, Warner JO. Bronchopulmonary dysplasia: the outcome. *Arch Dis Child* 1987;**62**:1099–101.
[17] Hoshi J, Nishida H, Arai T, *et al.* Clinical and etiological analysis of six cases with early onset type of Wilson-Mikity syndrome. *Acta Neonatologica Japonica* 1987;**23**:853–7.
[18] Ohde S, Nagoshi R, Suzuki K, *et al.* Clinical study of chronic lung disease. *Acta Neonat Jap* 1988;**24**:464–72.
[19] Aherne WA, Cross KW, Hey EN, *et al.* Lung function and pathology in a premature infants with chronic pulmonary insufficiency (Wilson-Mikity syndrome). *Pediatrics* 1967;**40**: 962–74.
[20] Coates A, Bergsteinsson H, Desmond K, *et al.* Long-term pulmonary sequelae of the Wilson-Mikity syndrome. *J Pediatr* 1978;**92**:247–52.
[21] Krauss AN, Levin AR, Grossman H, *et al.* Physiologic studies on infants with Wilson-Mikity syndrome: ventilation-perfusion abnormalities and cardiac catheterization angiography. *J Pediatr* 1970;**77**:27–36.
[22] Ogden BE, Murphy SA, Saunders GC, *et al.* Neonatal lung neutrophil and elastase/proteinase inhibitor imbalance. *Am Rev Respir Dis* 1984;**130**:817–21.
[23] Janoff A. Granulocyte elastase: role in arthritis and in pulmonary emphysema. In: Havemann K, Janoff A, eds. *Neutral proteases of human polymorphonuclear leucocytes.* Baltimore: Urban and Schwarzenberg, 1978:391–418.
[24] Fujimura M. Granulocyte elastase in the tracheal aspirate of low birth-weight infants. *Japanese Journal of Pediatrics* 1987;**40**:339.

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Are preterm infants at risk for subsequent fractures?

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SUMMARY In Cambridge, between 1979 and 1988, the prevalence of low gestation (<37 or <33 weeks) was not increased in 362 children under 6 years presenting to casualty with fractures compared with that in 362 children presenting without fractures or in all inborn surviving babies. These data are relevant to the assessment of suspected child abuse after preterm birth.

Metabolic bone disease is common in preterm infants,[1] and is associated with decreased bone mineral content and an increased risk of fractures in the neonatal period.[2] There is little information, however, on the persistence of reduced bone mineralisation, and whether early bone disease results in an increased risk of fractures in childhood. Such information would be of importance both clinically, and medicolegally, in the assessment of cases of suspected child abuse.

We conducted a study to test the hypothesis that premature infants, especially those under 33 weeks' gestation (the high risk group for early bone disease) are at increased risk of childhood fractures compared with infants born at full term.

Subjects and methods

A retrospective study of the accident service and paediatric department records at Addenbrooke's Hospital, Cambridge, was conducted, with ethical approval. The incidence of prematurity (<37 weeks and <33 weeks) was compared in three study groups. The index group comprised children ≤5 years of age, born within the period 1979–87, and presenting to the accident service with a fracture. A comparison group included the next patient in the accident service register ≤5 years of age presenting for any reason other than fracture. All these infants were born in the Maternity Hospital in Cambridge, and therefore comprised a defined local population. A second comparison group included the entire population of surviving infants born at the Maternity Hospital within the same period. Gestational age in the first two groups was determined from obstetric records, based on maternal dates and early scans; and in the third from locally collected statistics, which showed that 6·8% of inborn infants were <37 weeks' and 1·1% were <33 weeks' gestation. Using the latter figures (which excluded in utero and neonatal regional transfers and neonatal deaths) we calculated a sample size of 700 subjects (divided equally between the index and accident service control groups) was required to detect a twofold increase in the proportion of children born preterm (<37 weeks) and a fourfold increase in those born at <33 weeks' gestation in the index group (for 5% significance at 80% power).[3] In the index cases further information was collected on fracture site and age at presentation.

Results

A total of 362 index and 362 control cases were collected from the accident service records, together with data from the 34 837 births at the Maternity Hospital for the period 1979–87.

Table 1 compares the incidence of prematurity (<37 weeks and <33 weeks) between the three groups. No significant difference in incidence of prematurity was found between these groups.

In the index group, there was no difference in fracture site between the gestational age groups (table 2). Mean age of presentation with fracture was similar between the full term and preterm populations, but tended to be lower in the small subgroup born at <33 weeks' gestation.

Table 1 *Incidence of prematurity in Cambridge inborn children ≤5 years of age (born 1979–87). Results are No (%)*

Gestational age (weeks)	*Index group (n=362)*	*Control group (n=362)*	*Inborn surviving infants (n=34 837)*
<37	23 (6·4)	26 (7·2)	2369 (6·8)
<33	4 (1·1)	3 (0·8)	393 (1·1)

Index group: children presenting to the accident service with fractures; control group: children presenting without fractures; inborn surviving infants: all babies inborn during the nine year study period.

Table 2 *Fracture site and mean age at presentation to accident service department for children ≤5 years of age*

Gestational age (weeks)	*Fracture site (No (%))*				*Mean (SD) age presentation at (decimal years)*
	Limb	*Skull*	*Other*	*Total*	
≥37	287 (85)	21 (6)	31 (9)	339 (100)	3·4 (1·6)
33–36	16 (85)	1 (5)	2 (10)	19 (100)	3·5 (1·7)
<33	3 (75)	1 (25)	0	4 (100)	1·5* (0·6)
Total	306 (85)	23 (6)	33 (9)	362 (100)	

*Compared with infants ≥37 weeks, $p<0\cdot05$.

Discussion

Premature infants have an increased susceptibility to fracture in the neonatal period.[2] This study suggests, however, that a substantially increased risk does not persist into childhood.

A twofold increase in the incidence of prematurity (<37 weeks) and a fourfold increase in the proportion of subjects <33 weeks' gestation in the fracture group has been excluded by our study. Furthermore, given the similarity in proportions of subjects <37 and <33 weeks' gestation in the index cases and controls, a new study, based on our data, would require a population of infants larger than that born in Britain each year to challenge the null hypothesis (no increased incidence of low gestation in fracture cases).

We emphasise, however, that the fractures described in this study were those that presented clinically; we cannot exclude the possibility that occult fractures had a differential incidence between preterm and term infants. Occult rib fractures are seen frequently in sick preterm neonates, though how long these persist is uncertain. For fractures presenting clinically, there was no difference in fracture site between infants born term or preterm.

Our failure to show an increased fracture risk in preterm infants does not necessarily imply that they have normal skeletal maturation. Indeed, data from this group suggest that early bone disease is strongly associated with impaired linear growth in the first two years.[4]

There was a trend towards a reduced age at presentation with fracture in the small subgroup of infants born at <33 weeks' gestation. This may have been a chance occurrence, and further investigation would require a larger sample.

Tensile strength of bone is related to bone mineral content, which has been found to be reduced in fractures in the elderly.[5] Interestingly, no such association has been found in investigations of childhood fractures,[6] raising the possibility they are more a reflection of a child's activity than due to defective mineralisation.

Our data indicate that preterm birth is not a major aetiological factor in childhood fractures. We suggest, therefore, that prematurity may not be an adequate explanation for a fracture in a case of suspected child abuse.

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References

1 Brooke OG, Lucas A. Metabolic bone disease in preterm infants. *Arch Dis Child* 1985;**60**:682–5.

2 Amir J, Katz K, Grunebaum M, Yosipovich Z, Wielunsky E, Reisner SH. Fractures in premature infants. *J Pediatr Orthop* 1988;**8**:41–4.

3 Pocock SJ. The size of a clinical trial. *Clinical trials: a practical approach*. Chichester: John Wiley, 1983:123–38.

4 Lucas A, Brooke OG, Baker BA, Bishop N, Morley R. High alkaline phosphatase activity and growth in preterm neonates. *Arch Dis Child* 1989;**64**:902–9.

5 Nilsson BE, Westlin NE. The bone mineral content in the forearm of women with Colles' fracture. *Acta Orthop Scand* 1977;**45**:836–44.

6 Cook SD, Harding AF, Morgan EL, *et al*. Association of bone mineral density and paediatric fractures. *J Pediatr Orthop* 1987; **7**:424–7.

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Aneurysm of the ductus arteriosus

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SUMMARY Two neonates with aneurysms of the ductus arteriosus were seen. One suffered a fatal spontaneous rupture of the aneurysm, and the second (in which it was an incidental finding) the aneurysm was monitored by echocardiography, and seemed to resolve spontaneously.

Aneurysm of the ductus arterious in infants is rare and potentially fatal, but often not recognised. It can present with common symptoms such as cough, hoarseness, and dyspnoea and should be included in the differential diagnosis of all mediastinal masses. The aims of this paper are to highlight the disease and to show the value of echocardiography in establishing the diagnosis. We hope that improved diagnosis will permit appropriate treatment to be instituted and fatal complications avoided.

Case reports

CASE 1

A 3 week old boy who had been previously well presented with a cough and hoarse cry. An upper respiratory tract infection was diagnosed and treated. His symptoms were stable for six days, but then deteriorated with increasing respiratory distress. On admission to hospital he was ill, cyanosed, and pale. He had a tachycardia, was tachypnoeic and had widespread crepitations in both lungs. A chest radiograph showed an enlarged heart and opacities in the left upper zone. Arterial blood sampling showed that he was hypoxic (oxygen saturation 76%), with a metabolic acidosis (pH 6·97), and a haemoglobin concentration of 98 g/l. Shortly after admission he had a cardiopulmonary arrest; he was resuscitated and transferred to the cardiac intensive care unit. He was moribund with a blood pressure of 30/20 mm Hg and his haemoglobin concentration had fallen to 42 g/l. A repeat chest radiograph showed complete opacification of the left hemithorax with the mediastinum shifted to the right.

Echocardiography was performed using the UM4 (ATL) echo/Doppler equipment. The intracardiac anatomy was normal. There was a moderate pericardial effusion and a large collection of blood in the left hemithorax. On the suprasternal view a thick walled cystic mass 3 cm in diameter was seen. It displaced the left pulmonary artery anteromedially (fig 1a), and the distal aortic arch posterolaterally (fig 1b).

The pericardial effusion was tapped, and 30 ml of

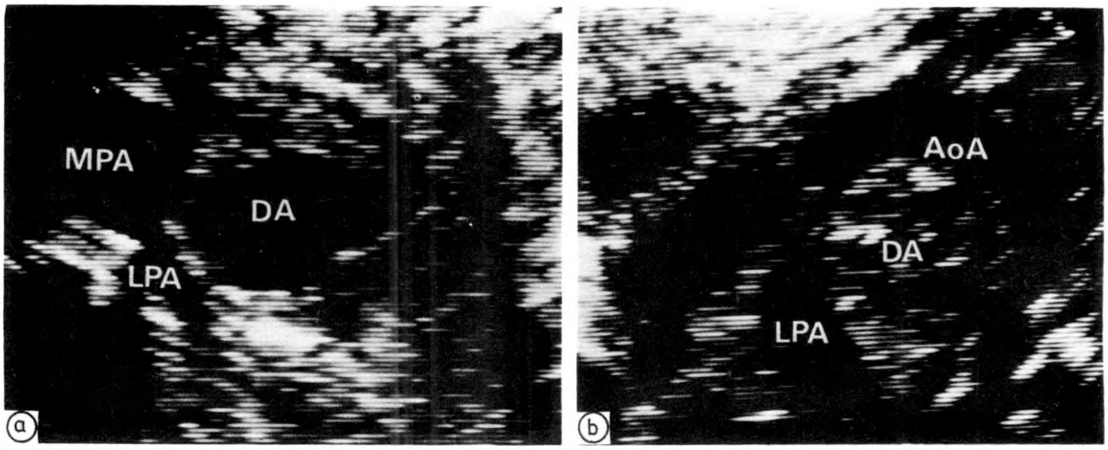

Fig 1 *(a) Suprasternal view showing left pulmonary artery (LPA) displaced anteromedially by the thick walled ductal aneurysm (DA). MPA=main pulmonary artery. (b) Posterolateral displacement of the aortic arch (AoA) by the ductal aneurysm (DA), with more than usual separation of the left pulmonary artery (LPA) and distal arch.*

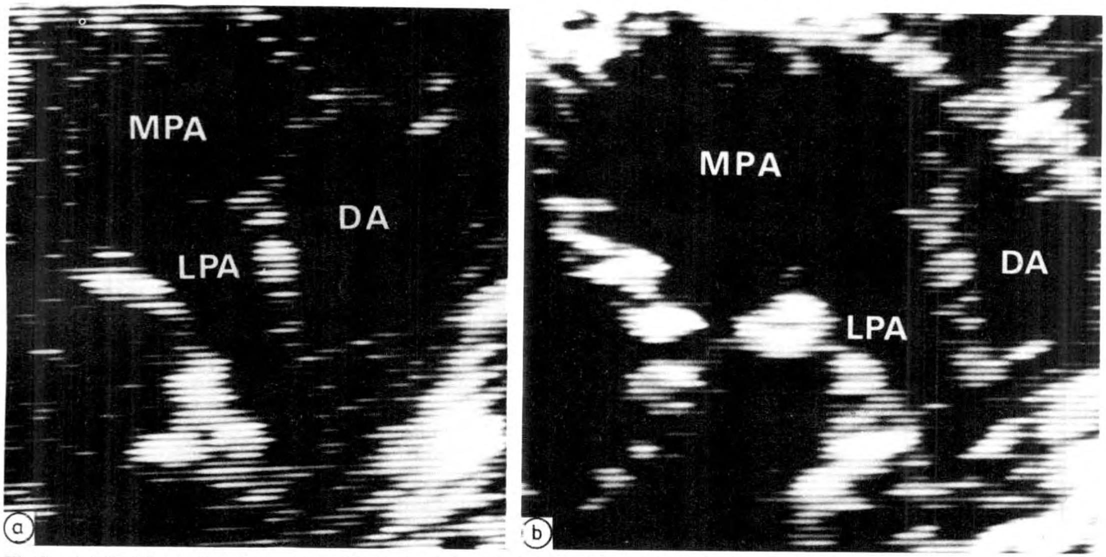

Fig 2 *(a) Fusiform ductal aneurysm (DA) posterolateral to the left pulmonary artery (LPA) and main pulmonary artery (MPA). (b) Repeat echocardiogram five days later. Note pronounced reduction in the size of the ductal aneurysm (DA) compared with the main pulmonary artery (MPA) and left pulmonary artery (LPA).*

haemoserous fluid was drained with a resulting temporary rise in blood pressure and reduction in pulse rate. The improvement could not be maintained, however, despite large infusions of colloid and blood. A left pleural tap drained fresh blood. Because the wall of the cyst was so thick (fig 1a) a bleeding mediastinal tumour was suspected and an emergency left thoracotomy was carried out. After evacuating a large amount of fresh blood clot there was exsanguinating haemorrhage from the region of the ductus arteriosus. The ductus had disintegrated and the aortic and pulmonary ends were open and bleeding. Attempts to control the haemorrhage failed. Histology of biopsy specimens taken from the region of the ductus showed laminated organised thrombus, which supported a diagnosis of ruptured aneurysm of the ductus arteriosus.

CASE 2

A baby boy was born at full term by emergency caesarean section for fetal distress. There was meconium stained liquor, profound bradycardia, and the baby made no respiratory effort. He was slow to respond to resuscitation and required continued intermittent positive pressure ventilation. On physical examination there was a left renal mass (later shown to be due to renal vein thrombosis) and a soft cardiac murmur. Chest radiograph showed bilateral upper zone opacification, worse on the left. Echocardiography showed normal intracardiac anatomy. There was pronounced fusiform dilatation of the ductus arteriosus (fig 2a) with flow from the aorta to the pulmonary artery shown on Doppler scanning, confirming ductal patency. At its widest diameter, the ductus was three times the size of the distal aortic arch. Daily echocardiograms showed progressive diminution in the size of the aneurysm, associated with closure of the pulmonary end (fig 2b). After a week the aneurysm was only slightly bigger than the distal arch, and there was no demonstrable flow shown on Doppler scanning. His clinical condition progressively improved and the left upper zone changes shown on chest radiography diminished in association with the reduction of the aneurysm size on echocardiography. Continued cardiac and renal surveillance is planned.

Discussion

These cases represent the extremes of a rare disease that is not often recognised. Spontaneous aneurysm of the ductus arteriosus is recognised in two groups: young infants and adults. The infant type is seen most commonly under 2 months of age and usually consists of a fusiform dilatation of the ductus with an open aortic end and a partially or completely closed pulmonary end.[1] It is usually an incidental finding at necropsy.[2] When symptoms occur they are commonly associated with recurrent laryngeal nerve palsy with cough, hoarse cry, and inspiratory stridor. Rarer presentations include phrenic nerve palsy or thromboembolic complications.[2 3] Spontaneous rupture and dissection has been described and was the probable cause of death in five of the 51 cases cited by Cruickshank and Marquis.[4] It has been assumed that aneurysm of the ductus may resolve spontaneously as thrombus within it becomes organised and fibrosed, but there is no documented evidence for this. In view of the possible fatal complications, therefore, surgical treatment is advocated. Heikkinen and Simila were the first to describe successful elective resection of the aneurysm in two neonates.[3]

Greater awareness of the condition could have facilitated a preoperative diagnosis in case 1. It was impossible to achieve vascular control at left thoracotomy but the use of cardiopulmonary bypass, hypothermia, and circulatory arrest would certainly have helped in achieving haemostasis. Review of reported work showed only one successfully treated ruptured aneurysm.[5] We recommend the use of cardiopulmonary bypass in suspected cases of aneurysmal rupture.

Because of our experience with case 1, the diagnosis was easily made with the use of echocardiography in case 2. The presence of a cystic mass in continuity with the left pulmonary artery and the aorta was unmistakable. Provided one maintains a high index of suspicion and includes aneurysm of the ductus in the differential of all mediastinal masses, the diagnosis can accurately and confidently be made on echocardiography. Further investigations, particularly angiography (which may jeopardise an already critically ill infant), are unnecessary.

In the second case the early natural history of the condition was documented. The rapid reduction in the size of the aneurysm led us to question the need for surgical intervention in all cases. A conservative policy of closely monitoring the clinical progress and echocardiographic appearances was adopted. If the aneurysm had not undergone rapid resolution or had increased in size we would have resected it because of the risks of endocarditis, ruputure, and compression of adjacent structures.

We recommend urgent operations using cardiopulmonary bypass if a ruptured aneurysm is suspected. If there is no evidence of rupture, however, we believe a conservative policy of monitoring the progress of the aneurysm by echocardiography is justified. If the aneurysm increases in size or does not undergo rapid resolution we recommend urgent resection.

Addendum

Since submitting this paper we have recognised two further cases. One resolved spontaneously and the other was corrected during cardiac surgery for pulmonary atresia.

References

[1] Heikkinen ES, Simila S, Laitinen J, *et al.* Infantile aneurysm of the ductus arteriosus: diagnosis, incidence, pathogenesis and prognosis. *Acta Paediatr Scand* 1974;**63**:241–8.

[2] Kirks DR, McCook TA, Serwer GA, *et al.* Aneurysm of the ductus arteriosus in the neonate. *AJR* 1980;**134**:573–6.

[3] Heikkinen ES, Simila S. Aneurysm of the ductus arteriosus in infancy: report of two surgically treated cases. *J Pediatr Surg* 1972;**7**:392–7.

[4] Cruickshank B, Marquis RM. Spontaneous aneurysm of the ductus arteriousus: a review and report of the tenth adult case. *Am J Med* 1958;**25**:140–9.

[5] Ferlic RM, Hofschire PJ, Mooring PK. Ruptured ductus arteriosus aneurysm in an infant – report of a survivor. *Ann Thorac Surg* 1975;**20**:456–60.

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Ciprofloxacin in neonatal Enterobacter cloacae septicaemia

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SUMMARY Multiresistant *Enterobacter cloacae* infection in six premature infants was eradicated with intravenous ciprofloxacin (10 mg/kg day). Bacterial resistance did not develop. Adequate plasma ciprofloxacin concentrations were achieved in all treated patients. No clinical evidence of side effects was observed.

Modi *et al* have documented an outbreak of multiresistant *Enterobacter cloacae* infection that was observed in this regional intensive care unit between July 1985 and January 1987.[1] The organism was resistant not only to penicillins and aminoglycosides but also to third generation cephalosporins. Later in 1987 a further six infants developed septicaemia due to the same multiresistant strain of *E cloacae* that showed in vitro sensitivity to ciprofloxacin (Ciproxin, Baypharm). Ciprofloxacin is a new synthetic fluoroquinolone with broad spectrum bactericidal activity and effective tissue penetration.[2] It has not been recommended for use in children as it has caused arthropathy in weight bearing joints of experimental animals. Chapman *et al* had previously treated *E cloacae* septicaemia with ciprofloxacin in a premature infant.[3] We report our experience of treating the six infants in the second outbreak of *E cloacae* infection with ciprofloxacin.

Patients and methods

CLINICAL ASPECTS

The six infants were treated in the regional neonatal intensive care unit, Liverpool Maternity Hospital. All infants had received at least two courses of different antibiotics before the ciprofloxacin. These included ampicillin, gentamicin, netilmicin, cefotaxime, and chloramphenicol. Ciprofloxacin 10 mg/kg/day in two divided doses was given intravenously for 14 days to infants 2–5 and 21 days in the case of infant 1 who had ventriculitis. Patient 6 died nine days after ciprofloxacin was started.

Serum concentrations were estimated after the third dose in all cases, and in the cerebrospinal fluid on two occasions in one infant.

SURVEILLANCE AND TREATMENT

Prevention practices and antibiotic treatment of babies in this nursery have been described previously.[1] The combination of ampicillin and gentamicin was used as a standard initial treatment of suspected infection.

LABORATORY METHODS

Isolation and identification of organisms as well as the breakpoint technique for determination of antibiotic sensitivities were used.[1] The breakpoints for ciprofloxacin were 0·5 mg/l (low) and 2·0 mg/l (high). The sensitivity of the strains of *E cloacae* isolated initially were tested by Stokes disc method[1] with disc concentration of 5 mg. For selected strains, the minimum inhibitory concentration of ciprofloxacin was determined. For this Adatabs (Mast House, Bootle) were used to make twofold dilutions ranging from 2 mg/l to 0·015 mg/l, a technique similar to the breakpoint method.[1]

Ciprofloxacin serum concentrations were measured by high performance liquid chromatography. Trough and peak plasma concentrations were measured, taking blood 30 minutes after intravenous injection for the latter.

Results

CLINICAL ASPECTS

All six infants were premature and of very low birth weight (see table 1). All infants were ventilated for chronic lung disease. Three infants had periventricular haemorrhages and two developed necrotising enterocolitis before the institution of ciprofloxacin treatment. The initial sites of colonisation with *E cloacae* are shown in table 1. There was a short interval between colonisation and the onset of systemic infection in the majority. Although *E cloacae* was eradicated in every case, three of the six infants died. The causes of death were necrotising enterocolitis and bronchopulmonary dysplasia in infant 3, bronchopulmonary dysplasia in infant 4, and necrotising enterocolitis in infant 6. In all infants *E cloacae* had been eradicated four to five days after starting ciprofloxacin.

Table 1 *Patient details*

Patient No	*Gestation (weeks)*	*Birth weight (g)*	*Site of isolation of E cloacae*	*Age at time of isolation (days)*	*Outcome and cause of death*
1	28	1200	Pharyngeal aspirate	16	
			Blood	24	
			Cerebrospinal fluid	26	Survived
2	28	800	Throat swab	29	
			Blood	29	Survived
3	24	800	Throat swab	25	Died aged 81 days of necrotising enterocolitis and bronchopulmonary dysplasia
			Blood	30	
4	26	900	Pharyngeal aspirate	6	Died aged 66 days of bronchopulmonary dysplasia
			Blood	6	
5	26	1000	Pharyngeal aspirate	5	
			Blood	5	Survived
6	29	700	Throat swab	6	Died aged 19 days of necrotising enterocolitis
			Blood	7	

Table 2 *Ciprofloxacin treatment*

Patient No	*Age at start of ciprofloxacin (days)*	*Ciprofloxacin concentration (mg/l)*		*Time for eradication of E cloacae at all sites with ciprofloxacin (days)*
		Plasma	*Cerebrospinal fluid*	
		3rd dose		
1	35	Pre 0·04	0·88	
		Post 1·45		
		5th dose		
		Pre 0·57		4
		Post 1·77		
		14th dose		
		Pre 0·52	1·45	
		Post 2·15		
		3rd dose		
2	42	Pre 1·0		2
		Post 3·1		
		3rd dose		
3	49	Pre 2·2		5
		Post 4·6		
		3rd dose		
4	9	Pre 0·6		4
		Post 3·3		
		3rd dose		
5	21	Pre 2·6		5
		Post 5·7		
		3rd dose		
6	10	Pre 1·1		4
		Post 2·9		

MICROBIOLOGICAL ASPECTS

Ciprofloxacin concentrations in serum and cerebrospinal fluid are shown in table 2. The mean concentration in the cerebrospinal fluid was 64% of the corresponding post dose concentration in the serum.

At the beginning of this outbreak two babies were colonised and infected with strains sensitive to cefotaxime and ceftazidime. Later, isolates from various sites became resistant to these antibiotics. The isolates of *E cloacae* that subsequently colonised and infected other babies were resistant to cefotaxime and ceftazidime. The biotypes of all these *E cloacae* isolates were identical—that is, resistant to ampicillin, augmentin, trimethoprim, and cefuroxime. They had reduced sensitivities to aminoglycosides as shown by high breakpoints to gentamicin (4 mg/l), netilmicin (4 mg/l), and amikacin (16 mg/l). All isolates of *E cloacae* tested both by the disc and breakpoint method were sensitive to ciprofloxacin. The minimum inhibitory concentration for ciprofloxacin was 0·03 mg/l in four infants and 0·06 mg/l in two infants. There was no change in minimum inhibitory concentration during treatment with ciprofloxacin.

E cloacae was eradicated from all infants treated with ciprofloxacin. The median time for eradication was four days with a range of two to five days. After the use of ciprofloxacin, *E cloacae* has not been isolated again from infants at this hospital.

Discussion

As in previously reported outbreaks of *E cloacae* infection,[1] the strains causing neonatal infections were multiresistant. In this new outbreak the isolates of *E cloacae* were initially sensitive to third generation cephalosporins. Subsequent isolates, however, became resistant to cephalosporins, confirming our previous findings and supporting the possibility of a spread of a derepressed mutant.[1] These resistant strains of *E cloacae* were eradicated with ciprofloxacin treatment, previous courses of other antibiotics having failed. Resistance to ciprofloxacin was not encountered in our series, although Scully *et al* have described the development of resistance to ciprofloxacin in pseudomonas infection.[4]

By acting directly on the DNA gyrase enzyme that is present in many Gram negative bacteria, cell division is inhibited and plasmid mediated resistance is thus prevented.[2] Resistance to ciprofloxacin can only develop by bacterial mutation.

Eradication of the organism was achieved with a mean post dose concentration of 3·1 mg/l. We aimed for post dose concentrations below 7·0 mg/l.

Ciprofloxacin penetrates tissues well and therapeutic concentrations in the cerebrospinal fluid have been achieved even in subjects without inflammation of the meninges.[5] Isaacs *et al* have already reported the successful treatment of ventriculitis caused by a multiresistant *Pseudomonas aeruginosa* strain.[6] Concentrations of ciprofloxacin in the cerebrospinal fluid have been reported to be 40% of concentrations in serum in meningitis.[5] In our patients 60–70% of peak plasma concentrations were achieved in the cerebrospinal fluid—14 times the minimum inhibitory concentration of the organism. The ventriculitis was effectively treated with intravenous ciprofloxacin.

Unwanted effects of ciprofloxacin include damage to weight bearing joints, hepatic and renal impairment, interaction with DNA of lymphocytes and with theophylline. No side effects were observed in our patients.

The high mortality in this series was disappointing, although the delay between eradication of the organism and death suggested that death was not directly related to *E cloacae* infection. Despite the relatively late introduction of ciprofloxacin during the course of the infection the organism was eradicated in all cases.

With the benefit of this experience we would now use ciprofloxacin at a much earlier stage of the infective process, but only when our usual first line antibiotics are ineffective. We would advise caution when considering its use, however, and suggest that it should be prescribed only in instances of proved infection with multiresistant organisms.

We thank Mr R Whitaker for organising and supervising sensitivity testing to ciprofloxacin and Mrs S Longworth for typing this paper.

References

[1] Modi N, Damjanovic V, Cooke RWI. Outbreak of cepahlosporin resistant Enterobacter cloacae infection in a neonatal intensive care unit. *Arch Dis Child* 1987;**62:**148–51.

[2] Ciprofloxacin—an important new antimicrobial. *Drug Ther Bull* 1987;**25:**69–72.

[3] Chapman ST, Speller DCE, Reeves DS. Resistance to ciprofloxacin. *Lancet* 1985;**ii:**39.

[4] Scully BE, Neu HC, Parry MF, Mandell W. Oral ciprofloxacin therapy of infections due to Pseudomonas aeruginosa. *Lancet* 1986;**i:**819–22.

[5] McLain JB, Rhoads J, Kroll G. Cerebrospinal fluid concentrations of ciprofloxacin in subjects with uninflamed meninges. *J Antimicrob Chemother* 1988;**21:**808–9.

[6] Isaacs D, Slack MPE, Wilkinson AR, Westwood AW. Successful treatment of Pseudomonas ventriculitis with ciprofloxacin. *J Antimicrob Chemother* 1986;**17:**535–8.

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Fetal ascites: an unusual presentation of Niemann-Pick disease type C

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SUMMARY Two infants were seen with severe ascites detected before birth, a previously unreported presentation of Niemann-Pick disease type C. In the second infant no diagnostic storage cells were present in bone marrow. Confirmatory investigations were prompted by experience of the first case.

Niemann-Pick disease type C is a heterogeneous neurovisceral storage disorder inherited in an autosomal recessive fashion. A defect in cholesterol esterification has been identified, so that antenatal diagnosis is possible.[1]

Three presentations have been described. Some patients present with jaundice and hepatosplenomegaly in the first two months of life, most of whom become symptom free, though a few die of liver failure by the age of 6 months. Others have asymptomatic splenomegaly in the first six years of life, and yet others develop progressive neurological dysfunction or psychosis, with or without splenomegaly, between the ages of 2 and 59 years.

Progressive neurological deterioration occurs in all cases, often causing death by the third decade. Diagnosis is suspected on identifying typical storage cells in bone marrow and confirmed by electron microscopy of neural tissue; in addition a laboratory test has recently been described.[1]

We present two cases with severe fetal ascites, which is a previously unreported presentation.

Case reports

Both were girls and were first born infants of healthy, unrelated white parents without family histories of inherited disease. In both, other causes of fetal ascites and metabolic disorders associated with liver disease in infancy were excluded by appropriate investigations.[2] [3] In both electron microscopy of rectal suction biopsy specimens showed pleomorphic lamellar cytoplasmic inclusions in neurones. Laboratory studies using cultured skin fibroblasts showed considerably decreased intracellular esterification of exogenous non-lipoprotein 3H cholesterol at 0·95% (case 1) and 2·5% (case 2) of total cholesterol (normal range 7–13%). Intracellular esterification of exogenous lipoprotein-derived cholesterol (which was tested only in case 2) was also low (165 pmol/l; normal range 1210–2910 pmol/l) (M Vanier, personal communication).

CASE 1

Fetal ascites was detected ultrasonically at 18 weeks' gestation. At birth at 33 weeks' gestation the infant weighed 1930 g and had pronounced hepatosplenomegaly and ascites. Conjugated hyperbilirubinaemia developed on day 2, reaching a maximum value of 282 mmol/l on day 4 and gradually decreasing thereafter.

On day 39, when transferred to King's College Hospital she had severe cachexia, respiratory difficulty, and gross ascites, and had not gained weight. Serum bilirubin was 41 μmol/l (normal value <20), γ glutamyltransferase 115 IU/l (normal value < 100), and alkaline phosphatase 341 IU/l (normal value <260). Aspartate aminotransferase activity, albumin concentration, and prothrombin time were normal. Bone marrow contained storage cells typical of Niemann-Pick disease type C. No liver biopsy was carried out.

On medium chain triglyceride formula (Pregestimil, Mead Johnson), supplemented with Maxijul (Special Hospital Supplies) and restricted to 130 mg/kg/day, her dyspnoea and ascites cleared over 12 weeks, and she started to grow satisfactorily. At 30 weeks of age a normal diet was started. At 17 months her height and weight were at the 50th percentile for her age. Mild hepatosplenomegaly persisted. Development was normal.

CASE 2

Ultrasound scan at 33 weeks' gestation showed ascites. At 34 weeks fetoscopy was performed with insertion of abdominal amniotic drains. These became displaced, and she was born two days later weighing 2240 g, with pronounced hepatosplenomegaly, ascites, and respiratory difficulty. Jaundice developed on day 1, and bilirubin concentration reached a maximum of 400 μmol/l (65% conjugated) on day 13 when she was transferred to this hospital.

Aspartate aminotransferase activity was 117 IU/l and prothrombin time 33 seconds (control 14 seconds). Alkaline phosphatase and γ glutamyltransferase activities, and albumin concentrations were normal. Bone marrow aspirate showed numerous vacuolated macrophages, and examination of a liver biopsy specimen showed severe parenchymal liver damage with extensive cell loss and pericellular fibrosis. No storage cells were identified. Death from liver failure occurred at 19 days of age.

Discussion

Fetal or neonatal ascites has not previously been reported in Niemann-Pick disease type C, although it has been seen in other inherited storage disorders.[3] Although there is at present no treatment, identification is essential for genetic counselling and antenatal diagnosis. The prognosis is grim compared with that of cryptogenic hepatitis of infancy.[4] Bone marrow appearances led to the diagnosis in case 1, but were inconclusive in case 2. Suction rectal biopsy and fibroblast culture were carried out only because we had been alerted by the first case. For practical purposes the initial screening test at present must remain the presence of storage material in the bone marrow. Case 2 highlights the need for a readily available specific biochemical diagnostic test. Case 1 will eventually develop features of neural impairment for which there is no treatment. Liver transplantation has not prevented neurological deterioration in such patients and abnormal storage material accumulates in the transplanted liver.[5] Bone marrow transplantation that corrects the biochemical abnormality and arrests progression of Niemann-Pick type B disease and other complex storage disorders has not been reported in Niemann-Pick disease type C. If laboratory means of monitoring the disorder can be developed it may encourage bone marrow transplantation in Niemann-Pick disease type C even though the underlying biochemical abnormality remains unknown.

We would like to acknowledge the help of Drs M Vanier, B Portmann, R Ireland, and A H Fensom in investigating these cases.

References

[1] Vanier MT, Wenger DA, Cowley ME, Rousson R, Brady RO, Pentchev PG. Niemann-Pick disease group C: clinical variability and diagnosis based on defective cholesterol esterification. A collaborative study based on 70 patients. *Clin Genet* 1988;33: 331–8.

[2] Mowat AP. Hepatitis and cholestasis in infancy: intrahepatic disorders. *Liver disorders in childhood.* 2nd ed. London: Butterworths, 1987:37–88.

[3] Mowat AP. Inborn errors of metabolism associated with disordered liver function or hepatomegaly. *Liver disorders in childhood.* 2nd ed. London: Butterworths, 1987:166–209.

[4] Mowat AP. Idiopathic hepatitis (hepatocellular jaundice of infancy). *Liver disorders in childhood.* 2nd ed. London: Butterworths, 1987:61–4.

[5] Gaitner JC Jr, Bergmann I, Malatack J, *et al.* Neurovisceral storage disease with supranuclear ophthalmoplegia following orthoptic liver transplantation. *Pediatrics* 1986;77:104–6.

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Personal practice

Follow up studies: design, organisation, and analysis

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The follow up of infants who were exposed to adverse perinatal events is an important component of perinatal audit. We need to know whether the changes in perinatal care that have undoubtedly resulted in their improved survival have altered the incidence of impairments in their long term health, growth, or neurodevelopmental state. Moreover, recent improvements in diagnostic imaging techniques carried out in the neonatal period may considerably enhance our ability to predict longer term outcome.[1-3] Their importance lies not only in their use to monitor the eventual outcome for individual children, but because therein could lie some explanation of the mechanisms linking clinical pathological entities to the lesions visualised.[4]

In the absence of reliable validated routine health surveillance of all children, special studies are required to monitor the effects of rapidly changing perinatal practice and to evaluate specific components of care. Studies from any one centre are often too small to answer the questions being posed. The purpose of this paper is to discuss ways in which the design of following studies could be made compatible to enable multicentre collaboration, and thus provide more rapid feedback to neonatal paediatricians on current issues.

Background

In 1981, Kiely and Paneth outlined some of the theoretical problems that beset the design and reporting of follow up studies.[5] In the same year, Sinclair *et al* expanded on the methodological requirements for a proper evaluation of neonatal intensive care.[6] This included testing of the efficacy of particular treatments, their effectiveness in the field, the efficiency of these treatments in relation to other calls on resources, and the implications (economic and otherwise) of making them available to all who need them. In this paper we discuss some of the other issues and practical problems encountered in mounting follow up studies and suggest some solutions, but our main aim is to stimulate further discussion that will eventually encourage more effective use of the vast amount of resource and expertise expended in the name of 'follow up'.

How is the information derived from follow up studies used?

Most reported follow up studies based on hospital populations are descriptive or observational; that is, information is collected on the outcome of cohorts of children with particular characteristics—for example birthweight groups, infants who were ventilated, infants with seizures, and so on. This information tends to be misused in two ways. Firstly, associations between perinatal variables and outcome are observed, and there is then a tendency to ascribe—erroneously—a causal relationship between the two. Descriptive follow up studies can, however, only generate hypotheses that need to be tested in a new cohort. Causal relationships can be determined only by a randomised study design. Secondly, the information collected is compared with other similar observations made in previous years or in other centres. Differences in outcome are ascribed to differences in care, but they may merely reflect differences in the study population (either over time or between centres), or a lack of uniformity in the way outcomes have been defined or ascertained.

How can we improve on study design?

The design of the study must be prospective, with the aims, the population, and the outcomes defined before collection of data starts. The selection of a comparison group must also be considered.

DEFINITION OF THE AIMS

The aim of the study should be defined at the outset and usually falls into one of the following categories: to estimate the incidence of disease, disorder, or impairment in a defined cohort followed up for a specific period; to evaluate the effectiveness or otherwise of a particular therapeutic intervention; or to test a specific aetiological hypothesis.

A descriptive follow up study of the first type can provide important information on the natural history and prognosis of a condition, and can identify the presence or absence of associations between perinatal events and outcome. The evaluation of specific treatments is best achieved by randomised clinical trials with predefined measurements of outcome made by investigators who are preferably 'blind' to the original treatment. The role of each type of study is illustrated in the following example.

A study of the natural history and prognosis of a group of infants with neonatal seizures identified a subgroup of infants born at full term whose seizures occurred in the first 48 hours of life and who had sustained an intrapartum insult.[7] This gave rise to the aetiological hypothesis that changes in intrapartum management might reduce the number of infants born at full term with early seizures. It was then postulated that the rate of such seizures might provide a useful marker of the quality of intrapartum care.[8] Two case control studies were set up to investigate the relationship between various adverse outcomes of pregnancy including early neonatal seizures and the quality of care in pregnancy and labour; both showed that there was a strong association between suboptimal care and early seizures.[9] [10] The hypothesis was sustained in a large randomised controlled trial of intrapartum electronic fetal heart rate monitoring.[11]

DEFINITION OF THE STUDY COHORT

The criteria by which the study cohort is selected should be clearly defined and will be determined by whether the study is investigating prevalence, or the effects of an intervention, or testing a specific aetiological hypothesis.

In a study of the prevalence of morbidity, the population should be geographically defined.[5] This permits evaluation of care to a total population whose characteristics can be defined, and hence the findings can be compared with other populations for which reference statistics are available, taking account of any differences in these variables. Results from tertiary referral centres are difficult to interpret, because they are confounded by the transfer of mothers and infants between units. An example of the importance of this source of bias was described by Horwood *et al* who showed that 28% of the reduction in mortality experienced after the opening of a new intensive care centre was accounted for by the transfer in from outside the geographical region of mothers who had been thought, incorrectly, to be at high risk of a poor outcome.[12]

Enrolment in such a study can be on the basis of all births, or all live births. It is, however, important to ensure that a birth is also defined by some other variable such as—for example, all births over 500 g—because there is marked variation in the definition of live birth both internationally and within countries.[13] For the purposes of international comparison, it is preferable to use standard categories such as those recommended by the World Health Organisation for birthweight groupings.[14]

In the United Kingdom, information on all births to women resident in a defined geographical area can be obtained through statutory birth notifications that are held by district medical officers in England and Wales, and chief administrative medical officers in Scotland and Northern Ireland. In most district general hospitals outside the big conurbations, the difference between the hospital based population and the geographically based one is not large. Those infants who are in the geographical cohort, but who were not included in the hospital population, can be easily identified and included. In large cities where infants could be cared for in a wide range of tertiary care centres dictated by the availability of an intensive care cot, it is not possible to evaluate the quality of care from information on the outcome of infants treated in a single unit.

In conducting studies to test aetiological hypotheses, or intervention studies, it is not necessary to use a geographically defined population. Once the study cohort has been defined it is important that all are accounted for in the analysis. In the special case of an antepartum or intrapartum intervention study, the women entered into the study and all the infants born to those women should be included in the evaluation of the intervention. For example, in a trial of a tocolytic agent to prevent preterm labour, the condition of all the infants resulting from all the pregnancies being studied needs to be compared, whether they are born prematurely or not. It could—for example—be argued that postponing delivery might subject the fetus to additional unsuspected hazards.

DETERMINATION OF SAMPLE SIZE

The sample should be large enough to provide an answer, either on its own or in conjunction with other similarly structured studies, to the main question being asked in the study. The involvement of parents and children in a follow up study that has little chance of producing an answer to the question posed should be regarded as unethical.

In a study of prevalence in a defined population, the study size is obviously constrained by the available eligible entrants. In testing a hypothesis it is important that the sample size is big enough to permit identification of enough subjects with the predefined outcome. For example, there is little point in examining 100 infants for the incidence of an outcome that occurs in only 1/1000. Furthermore, the sample needs to be large enough to detect intergroup differences that are also biologically important.

It is difficult to give general advice because each study has a different perspective; a statistician should be consulted to advise on the necessary calculations based on the reported frequency of a given dichotomous outcome, or some measure of central tendency and dispersion such as the mean and standard deviation of a continuous outcome measure such as growth or a developmental score.[15] It should be borne in mind that the standard deviation for a developmental score is likely to be greater in a 'high risk' population than in the standardisation sample for the test.

In order to study infrequent outcomes (such as retinopathy of prematurity) or a limited population (such as infants weighing less than 1000 g at birth) within a reasonable time span, multicentre collaboration is essential to achieve sufficient numbers. For this to be successful, standard methods for identifying and reporting those outcomes are needed.

DEFINITION OF OUTCOMES

The outcomes to be measured will depend on the aim of the study, but whatever they are they must be specified and clearly defined before the start of the study. The outcome should be relevant to the child or family, or likely to improve our understanding of the aetiology or treatment of the condition being studied. The availability of funds or personnel, or both, may impose constraints on the study, making the choice of the most important and valid outcome measures even more essential.

Commonly reported outcomes are death, neurodevelopmental state, sensory impairment, morbidity, growth, behavioural attributes, and learning ability. It is important that the outcome to be measured can be defined accurately. In the case of death, for example, the definition is simple, whereas neurodevelopmental impairment or emotional problems would require careful definition before a study was undertaken.

In making a full evaluation of the effects of any treatment it is important to consider outcomes that have economic implications. Measures such as the need for hospital admission, length of stay, the need for increased primary health care contacts, and the use of ancillary services are important factors that could influence whether recommendations based on the findings of a particular study are accepted.

Measuring outcomes

The tests (tools) used in measuring outcome should have both reproducibility and validity. These terms are illustrated in the figure (JC Sinclair, personal communication).

Even with standardised 'tests' inaccuracies, imprecision, and biases can arise. There is a real chance of interobserver variation—and also intraobserver variation—and these need to be tested in a pilot study. It has been shown that further training can help to improve the level of agreement between observers.[16] The problem is accentuated when non-standardised tests, sometimes devised by the examiner, are used before being subjected to rigorous evaluation. There is a large number of tests currently in use for assessing a wide variety of functions at differing ages and we do not intend to discuss specific ones here. There is, however, a need for some consensus on the optimal ages for testing children and the most appropriate tests at each age in order to achieve comparability between studies.

Outcome measures that require intensive assessment by highly trained personnel are not feasible if the study population is large. Conversely, smaller studies on their own, or 'nested' in large trials, may provide the opportunity for in depth examination of unsuspected effects of the treatments being compared.

Other sources of outcome information

In addition to data collected in the study there are several other sources which can be used to find out about the longer term health and development of children. Information on deaths in the study cohort can usually be obtained from routinely collected data held by districts on the child health register, as the death of a child is entered immediately to ensure that parents are not contacted for routine appointments.

Where appropriate, for a small cost, the National Health Service record of individuals held by the Office of Population Censuses and Surveys can be 'flagged' so that the researcher will be informed of the death or emigration of study children, or their movement from one family practitioner committee area to another.

Parents, health visitors, general practitioners, community child health doctors, hospital paediatricians, and teachers as well as research workers

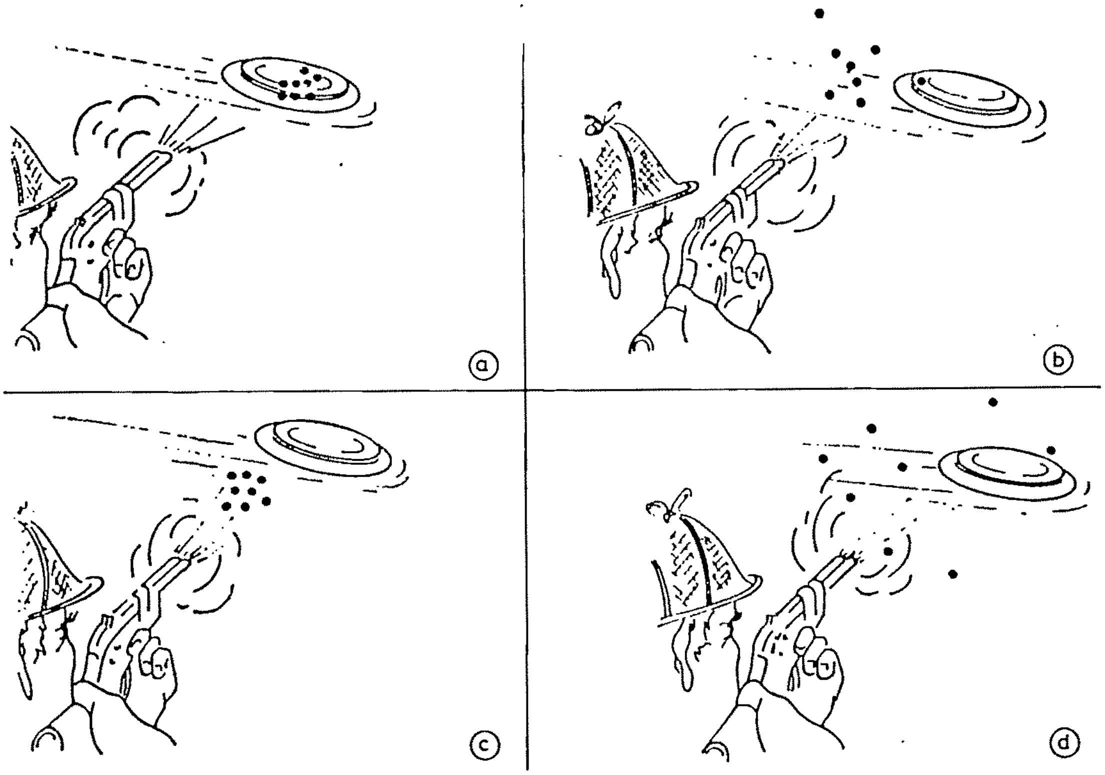

Figure *Reproducibility and validity: (a) the shot is valid (it hit the target) and reproducible (it hit the target every time); (b) one shot is valid, but the others went wide (not reproducible); (c) shots all hit the same spot (reproducible), but missed the target (not valid); and (d) shots neither valid nor reproducible.*

committed to the study can all provide their own perspectives on the child. The ideal, but expensive, situation would be to draw on all these sources, using them to validate each other. The advantages and disadvantages of the various sources are summarised in table 1. There is a need for validation of inexpensive methods of obtaining information on children's health and development. Such validation studies should be incorporated in more detailed and costly studies.

Reporting outcomes
In order to compare outcomes of studies from different centres the diseases, disorders, or impairments should be defined in a standard way.

It is helpful to consider outcomes in the way described in the *International Classification of Impairments, Disabilities and Handicap.*[17] A disease or disorder causes an impairment, which is defined as '. . . any loss or abnormality of psychological, physiological or anatomical structure or function'. A disability resulting from this impairment is '. . . any restriction or lack of ability to perform an activity in the manner or within the range considered normal for a human being'. Handicap is the disadvantage resulting from an impairment or disability that '. . . limits or prevents the fulfilment of a role that is normal (depending on age, sex, and social and cultural factors) for that individual'.

Impairments—Of these three terms, impairments are the most amenable to definition. For example, consensus has been reached on the standard description and reporting of the grades of retinopathy of prematurity.[18] A working group has developed a standard format for the recording and reporting of the motor deficit in cerebral palsy.[19] An attempt to provide uniformity in describing and identifying neurological impairments has been proposed by Amiel-Tison and Stewart.[20]

Table 1 *Summary of methods of follow up*

Sources of information	*Advantages*	*Disadvantages*
Routinely collected data (available in UK):	Inexpensive	Lack of uniformity between districts in child health surveillance
Preschool health (school health) system	Accommodate large study populations	
Hospital activity analysis		Limited detail
Parents:		
Parental questionnaires	Inexpensive Accommodate large study populations Unique knowledge of child's abilities and disabilities and social and behavioural attributes	Inaccuracy of recall 'Subjectivity' bias Inadequately validated
Health diaries	Improved accuracy of recall	'Subjectivity' bias Inadequately validated
Health visitors:		
Assessment	No additional funding if part of routine surveillance Can accommodate quite large numbers Specific training feasible	Additional workload Unstandardised 'tools' Interobserver variation
Teachers:		
Assessment	No additional funding if part of routine Can accommodate quite large numbers Use of standardised tests Explores cognitive development	Additional workload
Hospital based personnel:		
Routine outpatient	No additional visits	Lack of uniformity in testing Interobserver variation Limited time for assessment Clinical environment
Special study personnel:		
Standard protocol	Longer time for assessment Can be seen in own surroundings In depth study possible	Additional appointments Cost
Multiple observers	Larger numbers possible	Interobserver variation
Single observer	Relative consistency in observation In depth study possible Finer discrimination possible between groups	Small numbers only Cost Undetected systematic error

Disability—Assigning grades of severity to the disability arising from impairments is fraught with problems. In prevalence studies it is important that the 'cut off' point for ascertaining the condition under review is predefined for comparative purposes, as children with minimal problems may well not be universally recognised, and the reader needs to know the threshold of inclusion.

The description of disability needs to be related to a particular task and the developmental age of the child and must take account of the use and usefulness of aids.[21] In most currently reported studies, however, there is a lack of uniformity in describing disability in children and this is compounded by the arbitrary categorisation of disabilities into grades of 'mild', 'moderate', and 'severe', which may have different meanings in different studies. Furthermore, a physician's perception of the severity of a disability will depend on his or her clinical experience.

In a population based study in the Northern region of England a system was developed to categorise the degree of disability in a group of children with established cerebral palsy in which aspects of mobility, physical dependence, social and economic implications and resource use were summed to provide an overall measure of disability.[22]

In a recent study of disabilities in children by the Social Survey Division of the Office of Population Censuses and Surveys, considerable attention was paid to all these points; it will be a useful reference source.[23]

One possible way forward would be the development of a 'minimum data set' incorporating standard ways of categorising death, accepted definitions of impairments, and guidelines on assigning grades of severity. This would permit meaningful accurate comparisons between studies.

Handicap—This is a particularly difficult outcome for comparative use, as it depends heavily on subjective factors and is a reflection of the attitudes within the family and society as a whole to people with disabilities. It is best avoided in the types of study described here.

CHOICE OF A COMPARISON GROUP

In a controlled trial the control group should be randomly chosen from those eligible for entry to the study and therefore should be comparable with the index group in every respect other than the intervention being studied.

In an observational study there is no easy answer to the choice of a comparison group.[5] As previously stated, comparison of the outcome of hospital populations either over time (historical controls) or between centres may result in misleading inferences.

The choice of a comparison group depends on the question being asked. The choice must be between matched and 'unmatched' controls; if the preference is for matched controls, then subjects could be matched on antecedent factors such as maternal age, parity, birth weight, or gestational age, depending on the design of the study. In considering developmental outcomes, however, the over-riding determining factors are sex, social class,[24] and preschool experience,[25] so there is a rational basis for matching on those variables. Often it is better to match for only exceptional factors and adjust for others in the analysis. Overmatching can introduce difficulties in interpretation by taking out of consideration the very factor being investigated.

In a prevalence study in, for example, a very low birthweight population where the outcome to be measured is relatively uncommon, more than one control for each index case may be necessary to provide more accurate estimates of the increased relative risk experienced by the study subjects. But the advantage to be gained by increasing the number of controls soon falls off[15]; statistical advice is needed on this point.

The use of siblings as a comparison may have the advantage that socioeconomic variables and experience of parenting are likely to be the same. In some families, however, there will be no siblings and any finding limited to those cases who had a sibling would not necessarily be relevant for the one child family. Birth order in itself has been shown to have a significant effect on developmental score almost equal to that of the sex of the child.[26]

At an older age group, classroom controls of the same sex and social class are an attractive alternative, but this necessarily excludes children not able to attend school who may be represented to a greater extent in the index group.

If a concurrent comparison is carried out between two geographically defined populations with the same ascertainment criteria for measures of outcome, observed differences may indeed reflect differences in care; these may, however, also be the results of known differences in the sociodemographic structure of the populations, or to some as yet unidentified confounding factor. Information should be collected on factors known to be related to the outcome being measured, so that adjustment can be, made in the analysis for any marked imbalance.

TRACING CHILDREN

Tracing children can be time consuming, and several sources may need to be tapped.[27] High ascertainment rates are extremely important, because biases arise when follow up rates are low and it should be recognised that the lower the follow up rate the more inaccurate the estimate of the true rate of impairment. There is, furthermore, some evidence that children who are difficult to trace may be at a higher risk of an abnormal outcome. The extent to which this is true has recently been quantified in a study of very low birthweight children in the Northern region of England; children not seen on the first attempt had a sevenfold increased risk of impairment compared with those who were easily traced.[28]

If parents are aware from the time of birth or soon after that their children will form part of a study, contact can be maintained in a number of ways. In the 1946 British cohort used to study child health, birthday cards are used to keep in touch.[29] Assess-

Table 2 *Information required to tap sources helpful in tracing children*

Child:	Full name, date of birth, last address, National Health Service number, telephone number
Mother:	Full name, maiden name, National Health Service number
Father:	First name or initials
General practitioner:	Name, address
Grandparents:	Name, address, telephone number
Useful sources:	
Child health registers held by districts	
Family practitioner committees – registration departments	
National Health Service Central Registry in Southport (England), in Edinburgh (Scotland), and in Belfast (Northern Ireland)	
Telephone directories	
Local education authorities for children of school age	
Local post offices and electoral rolls	

ments in the early months of life can help in establishing rapport with the family. The information that can be helpful in tracing children within the United Kingdom, and agencies and other sources that can be used, are outlined in table 2.

In most follow up studies there are a few children who cannot be traced. Every effort should be made to restrict their number as much as possible It is important to record the number of untraced children and to compare their known characteristics with those of the children assessed.

Analysis and reporting of results

The selected study groups form the basis for the analysis. Statistical advice should be sought to ensure that the analysis of the data and interpretation of the results are appropriate, and this should be a stated part of the initial design of the study. There are several circumstances that deserve special consideration.

REFUSAL BY PARENTS TO PARTICIPATE

Parents may refuse because they suspect the motives of the assessor, particularly if their child is already the subject of concern on the part of the welfare authorities. A further explanation of the aims of the study will often secure their cooperation. In other cases parents may suspect that their child is not developing normally and do not welcome confirmation of their suspicions. It is also understandable that assessments may be unwelcome when family dynamics are disturbed, or where a child is severely impaired and already having regular assessments. Information from general practitioners and paediatricians may assist in these cases.

REFUSAL OF CHILDREN TO PARTICIPATE

Some children refuse to comply with testing. There is evidence that they do so because they are unable to do a test.[30] For those testing small children, it is often difficult to decide whether a child is failing to do a test or refusing to try it. Experience gained in a pilot study will help in defining a 'refusal'. Ounsted *et al* recommend exclusion of such children from the analysis of the variable concerned with a clear statement about their numbers and characteristics,[30] and Stewart (AL Stewart, personal communication) recommends assigning them to a grade above failure but below pass (for example, the 'suboptimal range' in the McCarthy test[31]).

EXCLUSIONS AFTER ASSESSMENT

It is difficult to make a general statement about this issue. In a randomised trial, once subjects have been assigned to a treatment they should all be included in the analysis. In an observational study of the developmental outcome of for example, children whose mothers had had some late onset pregnancy condition, then the inclusion of a child with a genetically determined syndrome would be inappropriate. When language development is a principal outcome of interest, then children whose first language differs from that of the tester are at a disadvantage, and not only in the sphere of language. Decisions about how to deal with these situations should be discussed at the outset of the study.

DISTRIBUTION OF CONFOUNDING VARIABLES

The first priority is to determine the distribution in the study group, and in the population from which the study sample was derived, of characteristics that might affect outcome such as birth weight, gestational age, sex, social class, birth order, and preschool experience.

DESCRIPTION AND CLASSIFICATION OF OUTCOME VARIABLES

The distribution of the outcome variables in the whole study group and separately in the comparison groups should be examined first. If the results in each group are normally distributed and have the same variance, comparison of means and standard deviations will be appropriate. Information on the number of subjects whose results fall below the 10th centile can be a useful additional way to describe the results especially if they are variables like developmental quotients or anthropometric measures. By itself, comparing groups of children falling below the 10th centile for a test may be a crude measure, and it is analogous to measuring the height of a group of people by placing a bar at the strategic height and seeing how many people could walk under it. Nevertheless, using cut off points of this type can also serve to increase the chance of data dependent choices to highlight or minimise apparent differences.

Where the results are skewed (that is, not normally distributed) statistical advice on the best and most informative approach should be sought. In the absence of a comparison group the findings can be described in terms of both means and standard deviations, and the numbers falling below the 10th centile.

Costing and funding

Follow up studies that do not use routine sources of information are usually costly to mount; they are usually project funded and estimates of their true cost are difficult to come by. It would be valuable for future researchers to have information on how

Table 3 *Costing a follow up study*

Personnel costs:
- Study design, including statistician's time
- Tracing children
- Training of personnel
- Conducting a pilot study
- Testing interobserver variation
- Organising appointments or questionnaires
- Assessing children
- Communicating findings to those responsible for clinical care
- Data entry and processing
- Data analysis (with computing and statistical advice)
- Writing up time

Administrative and clerical costs:
- Preparation of assessment protocols
- Stationery
- Photocopying, telephone, and postage

Equipment costs:
- Weighing and measuring equipment
- Kits for developmental tests
- Standard test recording forms
- Computer and other office equipment

Travel costs:
- Costs for either parents or study personnel

much a study has cost available as part of an appendix to publication. The items to be considered are outlined in table 3.

Estimates of travel costs depend on local circumstances and how far removed in age the subjects are from the age at entry into the study. For example, of a birth cohort contacted first at the age of 4 years, 80% were still living within 25 miles of the district general hospital in which they were born, 12% were between 25 and 100 miles away, and 1% were more than 200 miles away[25] (unpublished data).

Ethical perspective on follow up studies

It could be said that a physician has a responsibility to monitor the outcome of any treatment prescribed as part of routine health care: to ensure—for example—that a child's hearing is intact after treatment for an ear infection. The responsibility is perhaps less well defined in the perinatal period where the effectiveness of treatment for the mother is not always interpreted in the light of possible effects on the child.

The follow up systems developed by most special care nurseries provide a continuing clinical service for patients and their parents and have a counselling and supportive role as well as being a means of assessing overall health and development. Carefully designed follow up studies can be incorporated within these systems, but a fine balance needs to be achieved between fulfilling the needs of individual patients and meeting the rather stringent requirements of a research study.

Ethical approval must be sought for follow up where this is not considered an integral part of continuing clinical care. This is particularly important if a 'normal' control group is enrolled. Parents' consent should be sought after an explanation of the purpose of the study and what is entailed in the follow up assessment. This can be done orally or by letter, and parents can consent either by opting in or opting out.[32] General practitioners should be informed that their patient has consented to take part in a study. Results should be communicated promptly so that they are as relevant as possible to current practice; parents and general practitioners are entitled to receive the reports of assessments.

If we are concerned to monitor the effects of changing perinatal practice, then it must also be appreciated that the effect of follow up studies in themselves has never been evaluated fully. On the one hand, benefits may accrue from the early identification of—for example—sensory deficit and the implementation of treatment; on the other hand it may be that the identification of other problems of development, some of which cannot be 'cured', might cause unnecessary anxiety to parents and children. It is therefore important that studies should not be undertaken unless they are properly designed and of sufficient size to answer specific questions.[33] This counsel of perfection could well limit the number of studies but would undoubtedly increase their usefulness.

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References

[1] Stewart AL, Thorburn RJ, Hope PL, Goldsmith M, Lipscomb AP, Reynolds EOR. Ultrasound appearance of the brain in very preterm infants and neurodevelopmental outcome at 18 months of age. *Arch Dis Child* 1983;**58**:598–604.

[2] de Vries LS, Dubowitz LMS, Dubowitz V, *et al.* Predictive value of cranial ultrasound in the newborn baby: a reappraisal. *Lancet* 1985;**ii**:137–40.

[3] Stewart AL, Reynolds EOR, Hope PL, *et al.* Probability of neurodevelopmental disorders estimated from ultrasound appearance of brains of very preterm infants. *Dev Med Child Neurol* 1987;**29**:3–11.

[4] Bax MO. Forward from neonatology. *Dev Med Child Neurol* 1987;**29**:1–2.

[5] Kiely JL, Paneth N. Follow up studies of low birthweight infants: suggestions for design, analysis and reporting. *Dev Med Child Neurol* 1981;**23**:96–9.

[6] Sinclair JC, Torrance GW, Boyle MH, Horwood SP, Saigal S, Sackett DL. Evaluation of neonatal intensive care programs. *N Engl J Med* 1981;**305**:489–94.

7 Dennis J. Neonatal convulsions: aetiology, late neonatal status and long term outcome. *Dev Med Child Neurol* 1978;**20**:143–58.

8 Dennis J, Chalmers I. Very early neonatal seizure rate: a possible epidemiological marker of the quality of perinatal care. *Br J Obstet Gynaecol* 1982;**89**:418–26.

9 Niswander K, Elbourne D, Redman C, Henson G, Chalmers I, Macfarlane A. Adverse outcome of pregancy and the quality of obstetric care. *Lancet* 1984;**ii**:827–31.

10 Minchom P, Niswander K, Chalmers I, *et al*. Antecedents and outcome of very early neonatal seizures in infants born at or after term. *Br J Obstet Gynaecol* 1987;**94**:431–9.

11 MacDonald D, Grant A, Sheridan-Pereira M, Boylan P, Chalmers I. The Dublin randomised controlled trial of intrapartum fetal heart rate monitoring. *Am J Obstet Gynecol* 1985;**152**: 524–39.

12 Horwood SP, Boyle MH, Torrance GW, Sinclair JC. Mortality and morbidity of 500–1499 g birthweight infants liveborn to residents of a defined geographic region before and after neonatal intensive care. *Pediatrics* 1982;**69**:613–20.

13 Mugford M. International comparisons of definitions of vital events. *World Health Stat Q* 1983;**36**:201–12.

14 World Health Organisation. *International classification of diseases*. 9th Revision. Geneva: WHO, 1970.

15 Gore SM, Altman DG. *Statistics in practice*. London: British Medical Association, 1982.

16 Blair E, Stanley FJ. Interobserver agreement in the classification of cerebral palsy. *Dev Med Child Neurol* 1985;**27**:615–22.

17 World Health Organisation. *International classification of impairments, disabilities and handicaps*. Geneva: WHO, 1980.

18 International Committee for the Classification of Retinopathy of Prematurity. An international classification of retinopathy of prematurity. *Pediatrics* 1984;**74**:127–33.

19 Evans P, Johnson A, Mutch L, Alberman E. A standard form for recording clinical findings in children with a motor deficit of central origin. *Dev Med Child Neurol* 1989;**31**:119–27.

20 Amiel-Tison C, Stewart A. Follow up studies during the first five years of life: a pervasive assessment of neurological function. *Arch Dis Child* 1989;**64**:496–502.

21 Saigal S, Rosenbaum P, Stoskopf B, Milner R. Follow-up of infants 501–1500 g birthweight delivered to residents of a geographically defined region with perinatal intensive care facilities. *J Pediatr* 1982;**100**:606–13.

22 Jarvis S, Hey E. Measuring disability and handicap due to cerebral palsy. In: Stanley F, Alberman E, eds. *The epidemiology of the cerebral palsies*. Clinics in Developmental Medicine No 87. Spastics International Medical Publications. London: Heinemann Medical Books, 1984.

23 OPCS Surveys of Disability in Great Britain. Report No 3. *The prevalence of disability among children*. London: HMSO, 1989.

24 Ounsted MK, Osborn ML, Sleigh G, Good FJ. A method of developmental assessment at 4 years and some associated findings. *Early Hum Dev* 1979;**3**:1–19.

25 Dennis J, Johnson A, Mutch L, Yudkin P, Johnson P. Acid-base status at birth and neurodevelopmental outcome at four and a half years. *Am J Obstet Gynecol* (in press).

26 Morley R, Cole TJ, Powell R, Lucas A. Mother's choice to provide breast milk and developmental outcome. *Arch Dis Child* 1988;**63**:1382–5.

27 Hewitt A. In: Illsley R, Mitchell R, eds. *Low birth weight: a medical, psychological and social study*. Chichester: John Wiley, 1984:105–32.

28 Wariyar U, Richmond S. Morbidity and preterm delivery: importance of 100% follow up. *Lancet* 1989;**i**:387–8.

29 Douglas JWB, Blomfield JM. *Children under five*. London: Allen and Unwin, 1958.

30 Ounsted MK, Cockburn J, Moar VA. Developmental assessment at four years: are there differences between children who do or do not cooperate? *Arch Dis Child* 1983;**58**:286–9.

31 *McCarthy scales of children's abilities*. New York: Psychological Corporation, 1970.

32 Mutch L, King R. Obtaining parental consent – opting in or opting out? *Arch Dis Child* 1985;**60**:979–80.

33 Aylward GP. Developmental assessment: caveats and a cry for quality control. *J Pediatr* 1987;**110**:253–254.

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Regular review

Bone disease in preterm infants

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New work in bone physiology and cell biology during the last decade has made it possible to construct a model for the bone disease of preterm infants variously labelled 'rickets', 'osteopenia', and 'metabolic bone disease of prematurity'. The model proposed here explains its pathogenesis and its outcomes, and suggests a sequence of appropriate investigations as well as a scheme of management. The figure illustrates the basic processes of bone mineral metabolism in the perinatal period, and provides a framework against which the derangement in mineral homoeostasis leading to bone disease can be more clearly visualised.

Fetal bone mineral homoeostasis

Mineral accretion rates for both calcium and phosphorus increase throughout pregnancy, reaching a maxiumum during the third trimester of 3·0–3·7 mmol/kg/day for calcium and 2·4–2·7 mmol/kg/day for phosphorus.[1]

Fetal plasma calcitonin concentrations are also raised in utero[2]; though this peptide hormone is known principally for its hypocalcaemic action, there is substantial evidence that it has appreciable anabolic, mineral accreting effects on bone.

In contrast, the principal hormones mediating bone resorption in later life, parathormone, and 1, 25 dihydroxycholecalciferol, are found in low concentrations in the fetus.[2 3] Interestingly, however, prolonged low dose administration of these hormones in animals results in increased bone mass and thus both may be actively concerned in the accretion rather than resorption of mineral in utero.[4]

The first 48 hours

After ligation of the cord, the supply of calcium, phosphorus, and all other nutrients ceases abruptly. The continuing demand by bone for calcium entrains a rapid fall in blood calcium concentrations; the nadir for ionised calcium is usually at about 18 hours of age, slightly before that for total calcium.[5] In well preterm infants, hypocalcaemia will usually begin to improve by 24–30 hours of age, with values in the adult normal range attained by 48–60 hours. Factors unrelated to bone metabolism, particularly tissue hypoxia with subsequent calcium influx, may contribute to the low calcium concentrations seen in the sickest infants, in whom hypocalcaemia is more likely to persist and be more pronounced.

Previous workers have suggested, however, that the plasma parathormone concentration does not rise after birth, and that parathormone 'resistance' is likely to occur in preterm infants. Much of the early work on parathormone in the perinatal period was carried out using radioimmunoassays for either the carbon or the nitrogen terminal moiety of the molecule. Parathormone is an 84 amino acid peptide that is rapidly and continuously synthesised and almost immediately subjected to intracellular degradation.[6] Inactive fragments and intact molecules are stored together and then released, principally in response to hypocalcaemia. An increase in the amount of active hormone secreted could remain undetected, as the total terminal specific assay activity might not change appreciably. More recent studies, however, carried out with intact-molecule and active fragment (residues 1–34) assays, have shown a two fold to five fold increase in active parathormone secretion during the first 48 hours after birth.[5] The principal target organs for the parathormone molecules thus released are bone and kidney. In response to parathormone the kidney reabsorbs calcium and actively excretes phosphorus. A reasonable indication of the response to parathormone would therefore be to monitor urinary output of phosphorus over the first days after birth.

The longitudinal changes in whole blood ionised calcium up to the age of 72 hours, and in urinary phosphorus excretion up to the age of 5 days were studied in 18 preterm infants. High urinary concentrations of phosphorus on days 2 and 3 after birth were observed; subsequently, the phosphorus loss diminished rapidly and by day 5 phosphorus excretion had ceased in most of the infants studied

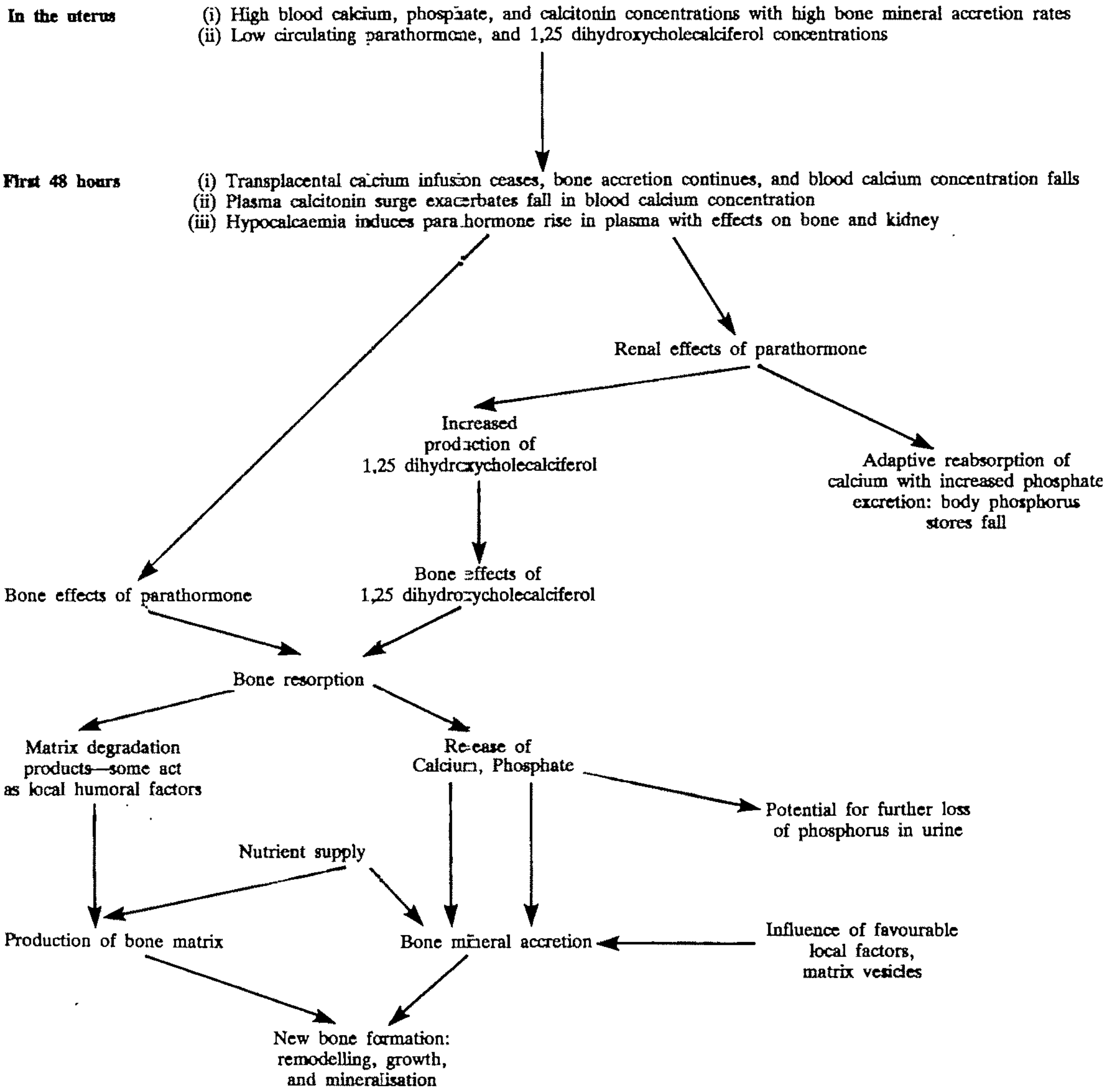
In the uterus
(i) High blood calcium, phosphate, and calcitonin concentrations with high bone mineral accretion rates
(ii) Low circulating parathormone, and 1,25 dihydroxycholecalciferol concentrations
First 48 hours
(i) Transplacental calcium infusion ceases, bone accretion continues, and blood calcium concentration falls
(ii) Plasma calcitonin surge exacerbates fall in blood calcium concentration
(iii) Hypocalcaemia induces parathormone rise in plasma with effects on bone and kidney
Renal effects of parathormone
Increased production of 1,25 dihydroxycholecalciferol
Adaptive reabsorption of calcium with increased phosphate excretion: body phosphorus stores fall
Bone effects of parathormone
Bone effects of 1,25 dihydroxycholecalciferol
Bone resorption
Matrix degradation products—some act as local humoral factors
Release of Calcium, Phosphate
Potential for further loss of phosphorus in urine
Nutrient supply
Production of bone matrix
Bone mineral accretion
Influence of favourable local factors, matrix vesicles
New bone formation: remodelling, growth, and mineralisation

Figure *Outline of processes of bone mineral metabolism in the perinatal period.*

(unpublished observations). These observations parallel results from animal studies of the effects of exogenously administered parathormone on phosphate metabolism in states of phosphorus repletion and depletion.[4]

Another important consequence of the action of parathormone on the kidney is the enhancement of 1,25 dihydrocholecalciferol synthesis. 1,25 dihydroxycholecalciferol is the most active metabolite of vitamin D_3 affecting not only the gastrointestinal absorption of calcium and phosphorus, but also the mobilisation of calcium from bone. 1,25 dihydroxycholecalciferol has a central role in the maintenance of calcium homoeostasis, which is discussed in detail below.

The release of parathormone is probably potentiated by the apparently paradoxical release of calcitonin almost immediately after birth.[7] In postnatal life calcitonin is secreted postprandially in response to gastrin production, and the initial surge of gastrin after the first feed may be responsible for the increase in plasma calcitonin concentrations seen at this time.[8] Calcitonin inhibits the resorptive response of osteoclasts and so delays the supply of calcium from bone to the circulation. The postnatal regulation of calcium homoeostasis is principally achieved by the interlocking actions of parathormone and 1,25 dihydroxycholecalciferol on bone, and by their separate effects on absorption and retention of mineral substrate.

Bone resorption

The isolation and culture of pure cell lines of osteoblasts and osteoclasts, have enabled rapid advances in our understanding of the underlying processes in bone.[9-11] It is now clear that osteoblasts and osteoclasts act together to undertake bone resorption, and that osteoclasts, having no parathormone receptors, exert resorptive activity in response to signals from osteoblasts. The specific effects of parathormone on bone are[4]: increased osteoblast permeability to calcium; release of collagenase from osteoblasts; and release of osteoclast activating factor(s) from osteoblasts, as a result of which osteoclasts increase in number and activity.

In addition, 1,25 dihydroxycholecalciferol, produced in response to the increased concentrations of parathormone, exerts synergistic effects on bone.[9 11] These are: activation of an osteoblastic calcium pump; increased activation and fusion of the monocyte/macrophage precursors of osteoclasts; production by osteoblasts of osteocalcin (Gla protein), which is chemotactic for osteoclasts; effects on immune cell function, particularly lymphocytes, with reduced interleukin 2, and increased interleukin 1 production, which enhances osteoclast formation and activity; and possibly a reduced response of osteoblasts to parathormone (reduced cyclic adenosine monophosphate response).

Thus parathormone and 1,25 dihydroxycholecalciferol have complementary actions; the increase in osteoblast permeability to calcium flux with the activation of a specific calcium pump provides an acute response to falling ionised calcium concentrations. The recruitment, activation, and fusion of osteoclast precursors, and their subsequent activity in response to osteoblast derived humoral factors, provides a longer term source of calcium.

New bone formation

The result of the resorptive process is to produce calcium, phosphorus, and breakdown products of bone matrix. These breakdown products are thought to act locally to promote new bone formation by osteoblasts. The increase in resorptive activity initiated by the surge of parathormone after birth is therefore matched by a concurrent increase in new bone formation. Though matrix volume is not reduced during this period of intense activity, net loss of bone mineral will occur if the exogenous supply of mineral substrate is inadequate.

In addition to the supply of adequate mineral substrate to normally functioning osteoblasts, a favourable local environment for bone mineralisation is also crucial to the remodelling and growth of bone; many factors have been identified in laboratory studies as having a role. In particular, there is a growing body of evidence to support the part played by matrix vesicles in the initiation and propagation of crystallisation.[12]

Matrix vesicles are discrete sacs that are derived from the osteoblast cell membrane. They are composed of a phospholipid bilayer rich in phosphatase enzymes including alkaline phosphatase, and they accumulate at the growing front of bone. At the pH of the mineralisation front, alkaline phosphatase functions principally as a phosphotransferase, transporting phosphate residues that have been cleaved by other phosphatase enzymes into the vesicle's sap.

Calcium enters the vesicle by diffusion, and is trapped by phosphatidyl serine. The additional accumulation of phosphate raises the saturation of the vesicle sap to the point where the calcium/phosphate solubility product is exceeded and crystallisation begins. Electron microscopic pictures have shown the growth of crystals on the inner leaflet of the vesicle that leads to its subsequent disruption as the ends of the crystal pierce the bilayered membrane.

These crystals then seed into the fluid at the mineralisation front and, given adequate mineral substrate there, act as foci for further crystallisation. The rate of turnover of matrix vesicles with the release of their membrane constituents, therefore, reflects the rate of initiation of crystallisation. Laboratory studies have shown that there are greatly increased numbers of matrix vesicles in rachitic growth plates[13]; this lends support to the concept that increased alkaline phosphatase activity in plasma may represent increased vesicle turnover in substrate or vitamin D deficient states.

During the early neonatal period the main determinants of bone remodelling, mineralisation, and growth are those that have been discussed in detail above. There are, however, many other factors affecting the fine control of bone homoeostasis,[9 11] of which two are of particular relevance to the preterm infant.

Aluminium is a potent inhibitor of bone mineralisation, and is present as a contaminant in parenteral nutrition solutions.[13] Up to 80% of the intravenously administered load may be retained, and significant deposition was found in bone after three weeks of parenteral feeding. It is possible that aluminium may exacerbate bone disease in preterm infants fed intravenously.

Immobility also causes loss of bone mass. Stress generated electrical potentials have been implicated in the osteogenic process, and prolonged periods of sedation or paralysis during mechanical ventilation increase the possibility of the loss of bone mass.

Mineral substrate insufficiency

Given an adequate nutrient supply, remodelling, mineralisation, and growth of bone should proceed normally in most infants. For bone disease to develop, depletion of mineral substrate must occur. Phosphorus depletion is likely to develop more rapidly as it may initially be lost in the urine, and protoplasmic metabolic requirements for phosphorus are greater than for calcium (extrapolating from data on fetal accretion rates and body composition studies, 0·6–0·7 mmol/kg/day compared with 0·2–0·3 mmol/kg/day).

Inadequate dietary provision of phosphorus—for instance, the exclusive use of unsupplemented human milk—compounded by the initial urinary phosphorus losses will result in low tissue phosphorus stores, and low circulating concentrations of phosphorus.

The reduced delivery of phosphorus to the kidney prevents further appreciable urinary losses and enhances renal production of 1,25 dihydroxycholecalciferol. The increase in circulating 1,25 dihydroxycholecalciferol in turn increases gastrointestinal absorption of both calcium and phosphorus. In addition, the release of parathormone is inhibited, further reducing the risk of phosphorus loss in the urine. As a corollary, however, renal reabsorption of calcium is reduced, with consequent hypercalcuria. The inhibition of parathormone release may also slow the process of bone reabsorption; nevertheless, the potent bone resorbing activity of 1,25 dihydroxycholecalciferol will continue to remove some calcium and phosphorus from bone.

In addition to the phosphorus absorbing and retaining processes detailed above, it is possible that hypophosphataemia is a key factor in accelerating directly or indirectly the turnover of matrix vesicles and hence increasing plasma alkaline phosphatase activity.

If mineral substrate provision continues to be inadequate, further substrate will be lost from bone in order to supply the needs of other tissues. The biochemical outcomes of these processes are interlinked; reduced concentrations of phosphate in urine and plasma precede the increasing urinary loss of calcium, and in extreme cases, hypercalcaemia. Raised plasma alkaline phosphatase activity is seen principally after 6 weeks of age.

Radiological and anthropometric changes occur slowly, being seldom evident before 6 weeks of age.[14 15] In the long term the principle outcomes for bone are linear growth, mineral content, and structural integrity. In a large study of preterm infants receiving different diets during the neonatal period, we found a significant association between the increase in plasma alkaline phosphatase activity and a reduction in height achieved at both 9 and 18 months implying that bone disease, reflected by increased remodelling activity during the first weeks of life, had a lasting effect on the infants' growth potential up to the age of 18 months.[16]

If these differences persist, then it is likely that the nutritional deprivation sustained by bone during this apparently critical phase of development has 'programmed' the bone to grow less slowly, as catch up growth would otherwise be observed when dietary sufficiency was achieved.[16]

The regulatory mechanisms for this adaptive change remain to be elucidated, but could involve changes in cell number, type, or function, either locally or systemically.

Investigation of early bone disease

Plasma phosphate concentrations fall gradually from 2 mmol/l to 1·0–1·5 mmol/l over the first week, and often reach a nadir during the second week after birth (unpublished observations). In infants depleted of phosphorus as a result of urinary losses and poor intake a further reduction to <1 mmol/l may occur, and this has been reported to be associated with the later development of biochemical and radiological evidence of bone disease. Urinary phosphate excretion initially may be increased but by day 5 is usually negligible. By contrast, urinary calcium losses increase and persist during the period that tissue phosphorus stores remain depleted. A prolonged absence of phosphate from the urine with persisting calciuria would imply continued tissue phosphorus depletion, and might be a useful marker to follow sequentially in an individual infant.

The natural history of plasma alkaline phosphatase activity is to rise over the first 3 weeks and plateau until the age of 5–6 weeks. Rises that occur after this are seen principally in infants with persistently low plasma phosphorus concentrations receiving low phosphate diets—for instance, unsupplemented human milk. Increased plasma alkaline phosphatase activity is widely quoted as being indicative of bone disease; difficulties arise in the interpretation of results and comparison with other centres because of the use of different assay systems with widely varying ranges and different units of measurement. Peak concentrations of greater than 7·5 times the maximum adult normal value for that particular alkaline phosphate assay have been associated with reduced linear growth velocity in the short term.[14] In the work previously referred to we found an area of demarcation at five times the maximum normal adult value for plasma alkaline phosphatase activity, with appreciable reductions in growth potential for infants with peak concentrations exceeding this limit.[16]

Radiological changes are usually not seen until the age of 6 weeks; reduced bone density, and abnormal bone remodelling in the form of cupping, splaying, and fraying of epiphyses may occur,[15] and—in extreme cases—there may be fractures of both ribs and long bones. The interpretation of radiographs is, however, subjective and the use of scoring systems has not improved their predictive value for minor to moderate degrees of demineralisation.

Photonabsorptiometry is a quick and accurate method of assessing sequentially the changes in bone mineral content at a specific site, usually the distal radius.[17] Photonabsorptiometry has shown that in infants receiving diets containing little mineral substrate, bone mineral content may remain unchanged, or even decrease initially, and then increase at a rate much less that attained in the uterus. By contrast, infants supplied with mineral in amounts approaching the intrauterine rate can maintain the fetal rate of mineral accretion.[18] The use of photonabsorptiometry is restricted to a few selected centres, however, and its principle use at present is for research rather than as an aid to diagnosis.

Radiographic densitometry is a low dose whole body technique that provides accurate information about the overall mineral state of the skeleton. As yet it has not been applied to preterm infants, but it could provide valuable data for body composition and mineral metabolism studies.

It has been often noted that peak alkaline phosphatase activity rarely occurs at the same time as radiological evidence of abnormal bone remodelling, or the degree of bone demineralisation as measured by photonabsorptiometry. This is essentially a reflection of the intrinsic properties of each investigation—plasma alkaline phosphatase activity is a measure of bone activity, possibly of the rate of mineral crystallisation; photonabsorptiometry gives an estimation of the amount of mineral actually in bone; and radiographs best show the abnormal remodelling resulting from an inadequate provision of mineral substrate for bones that are continuing to increase their matrix volume.

Short term anthropometry is useful as a non-specific adjunct to the radiological and biochemical investigations in that a reduced linear growth velocity at the age of 6 weeks would provide further evidence to support the diagnosis of bone disease.[16]

For practical purposes, sequential analysis of urinary calcium and phosphorus losses is likely to provide the earliest evidence of incipient metabolic bone disease. If by the age of 3 weeks calcium excretion is continuing, with no phosphorus appearing in the urine despite adopting the prophylactic measures outlined below, further mineral supplementation should be instituted.

Management

The management of this condition should essentially follow the dictum 'prevention is better than cure'. The degree and duration of mineral, and in particular phosphorus, depletion that will result in bone disease and the amount of supplementation that will prevent it are unknown. It is nevertheless possible to look at the provision of substrate by current feeding practices, formulate estimates of comparative bone mineral accretion rates, and so assess the minimum 'preventative' amounts of substrate intake required.

Unsupplemented human milk contains 0·5 mmol/100 ml of phosphorus. For infants receiving 200 ml/kg/day, and assuming 90–95% retention, 0·9–0·95 mmol/kg/day of phosphorus will be delivered. After allowing for basal protoplasmic requirements, approximately 0·3 mmol of phosphorus will be available for deposition in bone mineral. Calcium and phosphorus accrete at a ratio of 5:3 in bone; up to 0·5 mmol/kg of calcium might therefore be deposited—about 15% of the intrauterine accretion rate.

By contrast, a preterm formula containing 1 mmol/100 ml of phosphorus supplied at 180 ml/kg/day should result in a phosphorus retention of 1·6–1·7 mmol/kg/day. After allowing for protoplasmic requirements, about 1 mmol/kg/day of phosphorus is available for bone mineralisation, complexing with 1·6 mmol/kg/day of calcium—approximately 50% of the intrauterine accretion rate. Formulas with higher calcium and phosphorus contents are available in some countries, and have been used widely without adverse effects. Reported mineral accretion rates for infants fed these milks approach those achieved in the uterus,[18] but the reports are usually of well infants, fed fully by the enteral route by the age of 1 week. In addition, concern has been expressed generally that not all of the calcium and phosphate in these milks is available for absorption, possibly as a result of precipitation before feeding.

Given that protoplasmic requirements may be increased because of pre-existing tissue phosphorus depletion, particularly in infants who have previously been intravenously fed, the provision of 1 mmol/100 ml of phosphorus in milk given to preterm infants should be regarded as an absolute minimum.

The addition of phosphorus to expressed human milk is already common. Buffered neutral phosphate can safely be admixed with human milk over a 24 hour period to raise the phosphorus content from 0·5 mmol/100 ml to 1·0 mmol/100 ml.

Larger quantities of both calcium and phosphorus can be added to human milk. It is important to add the phosphorus salt (usually disodium phosphate) first so that it can enter the fat micelles. There is an appreciable risk of precipitation if calcium (usually given as calcium gluconate/glubionate) is added before the phosphorus.

As much as 1·6 mmol of phosphorus and 1·35 mmol of calcium can be added in this way.[19] Although some precipitation does occur, balance studies indicate that the absorption and retention of both calcium and phosphorus from such solutions is good.

The multinutrient fortifiers added to breast milk in some countries rely upon 'stabilising agents' to hold large quantities of mineral substrate in solution. Problems with precipitation and reduced absorption and retention have been reported, however, and in one study[20] it was found that retention of substrate from a preterm formula exceeded that from a fortified human milk solution. The use of such fortifiers is still the subject of intense research, and they cannot be unreservedly recommended for general use.

The addition of phosphorus to preterm formula is unlikely to be beneficial as the total amount of calcium retained and available for bone mineralisation is already completely utilised. Further addition of phosphate may in fact precipitate calcium from the milk solution, further reducing availability of the substrate.

Vitamin D_3 should be given routinely to all preterm infants.[21] For infants born in the United Kingdom whose mothers have received vitamin D supplementation during pregnancy the current recommendations are for 400–1000 IU/day, although it should be noted that with adequate dietary provision of calcium and phosphorus as little as 100 IU/day has been given without obvious adverse outcome for bone. For infants born to mothers not receiving vitamin D supplementation, as much as 1200 IU/day may be required. Increasing the supply of vitamin D_3 further may increase the short term retention of calcium, particularly in those infants in whom the supply of substrate is poor, but may also have longer term consequences for bone resorption and remodelling activities. In addition there may be as yet undefined consequences on cellular differentiation in other tissues; vitamin D_3 is a steroid type molecule, and a potent mitogenic agent.

Most preterm infants will not benefit from being given the active metabolites of vitamin D_3; these should be confined to those patients in whom a vitamin D_3 resistant state, for example, X linked hypophosphataemic rickets, has been confirmed.

Intravenously fed preterm infants are most at risk from poor supplies of mineral substrate. The solubility of calcium and phosphorus in parenteral nutrition solutions depends on a number of factors: the pH and the amino acid composition of the solution, the calcium salt used, and the temperature to which the solution is exposed.

The more acidic the solution, the greater the quantity of mineral substrate it can hold, and for most solutions calcium gluconate is more soluble than calcium chloride. Recently, amino acid solutions 'tailored' to the requirements of infants have been produced, with claims of improved capacity for calcium and phosphate. The testing of these solutions has not, however, taken account of the prolonged exposure to high temperatures (37°C for more than two hours) that may obtain in clinical practice.

The risk of precipitation with line blockage, or even microembolisation of crystalline particles, should not be ignored when using solutions with calcium and phosphate contents near the limit of solubility. The use of microporous filters within the line would reduce these risks appreciably. Using currently available amino acid solutions infused at 150 ml/kg/day, 1·1 mmol/kg/day of phosphorus and 1·5 mmol/kg/day of calcium can be administered at most. Claims of improved solubility and mineral delivery using glucose-1-phosphate have been made, but the use of an intermediary metabolite for this purpose is still under investigation.

In addition to problems with calcium and phosphorus delivery, the presence of high concentrations of aluminium in some solutions has given rise to concern about possible inhibition of osteoblastic function in infants receiving prolonged intravenous feeding.

In clinical practice, the infants likely to require mineral supplementation are those of less than 33 weeks' gestation. A reasonable 'prophylactic' measure would be the addition of 0·5 mmol of neutral phosphate solution to each 100 ml of expressed breast milk, or the use of a preterm formula containing at least 1 mmol/100 ml of phosphorus, and 1·75 mmol/100 ml of calcium. Supplementation should start as soon as enteral feeds are started, and continue until the infant achieves a weight of 2 kg or leaves the nursery. A urinary calcium: phosphorus ratio of >1 at the age of 3 weeks is an indication for further supplementation. For infants receiving preterm formulas, milks with higher mineral densities are available overseas, and may be introduced in the United Kingdom in the near future. Breast milk can be supplemented with both calcium and phorphorus using the previously described method.

It is hoped that early mineral supplementation will result in a reduction in the incidence of metabolic bone disease during the neonatal period, and maximise the potential for subsequent bone growth.

References

[1] Ziegler E, O'Donnel A, Nelson S, Fomon S. Body composition of the reference fetus. *Growth* 1976;**40**:329–41.

[2] Pitkin RM, Cruikshank DP, Schauberger CW, Reynolds WA, Williams GA, Horgis GK. Fetal calciotropic hormones and neonatal calcium homeostasis. *Pediatrics* 1980;**66**:77–82.

[3] Delvin E, Glorieux F, Salle B, David L, Varenne J. Control of vitamin D metabolism in preterm infants: fetomaternal relationships. *Arch Dis Child* 1982;**57**:754–7.

[4] Wong GL. Skeletal effects of parathyroid hormone. In: Peck WA, ed. *Bone and mineral research.* Vol 4. Amsterdam: Elsevier, 1986:103–30.

[5] Cooper LJ, Anast CS. Circulating immunoreactive parathyroid hormone levels in premature infants and the response to calcium therapy. *Acta Paediatr Scand* 1985;**74**:669–73.

[6] Cohn VD, Elting JJ. Synthesis and secretion of parathormone and secretory I protein by the parathyroid gland. In: Peck WA, ed. *Bone and mineral research.* Vol 2. Amsterdam: Elsevier, 1984:1–64.

[7] Romagnoli C, Zecca E, Tortorolo G, Diodato A, Fazzini G, Sorcini-Carta M. Plasma thyrocalcitonin and parathyroid hormone concentrations in early neonatal hypocalcaemia. *Arch Dis Child* 1987;**62**:580–4.

[8] Talmage RV, Cooper CW, Toverud SU. The physiological significance of calciton n. In: Peck WA, ed. *Bone and mineral research.* Vol 1. Amsterdam: Excerpta Medica, 1983:74–143.

[9] Sakamoto S, Sakamoto M. Bone collagenase, osteoblasts, and cell mediated bone resorption. In: Peck WA, ed. *Bone and mineral research.* Vol 4. Amsterdam: Elsevier, 1986:49–102.

[10] Mundy GR, Roodman GD. Osteoclast ontogeny and function. In: Peck WA, ed. *Bone and mineral research.* Vol 5. Amsterdam: Elsevier, 1987:209–80.

[11] Baron R, Vignery A, Horowitz M. Lymphocytes, macrophages and the regulation of bone remodelling. In: Peck WA, ed. *Bone and mineral research.* Vol 2. Amsterdam: Elsevier, 1984: 175–243.

[12] Anderson HC. Matrix vesicle calcification: review and update. In: Peck WA, ed. *Bone and mineral research.* Vol 3. Amsterdam: Elsevier, 1985:109–50.

[13] Sedman AB, Klein GL, Merritt RJ, *et al.* Evidence of aluminium loading in infants receiving intravenous therapy. *N Engl J Med* 1985;**312**:1337–43.

[14] Cooper PA, Rothberg AD, Pettifor JM. Early follow-up of very low birthweight infants after hospital discharge with respect to growth and mineral homeostasis. *J Pediatr Gastroenterol Nutr* 1988;**7**:577–82.

[15] Kulkarni PB, Hall RT, Rhodes PG. Rickets in very low birthweight infants. *J Pediatr* 1980;**2**:249–52.

[16] Lucas A, Brooke OG, Baker BA, Bishop N, Morley R. High alkaline phosphatase activity and growth in preterm neonates. *Arch Dis Child* 1989;**64**:902–9.

[17] Greer FR, Lane J, Weiners S, Mazess RB. An accurate and reproducible absorptiometric technioque for determining bone mineral content in newborn infants. *Pediatr Res* 1983;**17**:259–62.

[18] Chan GM, Mikur L, Hansen JW. Effects of increased calcium and phosphorus formulas on bone mineralisation in preterm infants. *J Pediatr Gastroenterol Nutr* 1986;**5**:444–9.

[19] Salle B, Senterre J, Putet G, Rigo J. Effects of calcium and phosphorus supplementation on calcium retention and fat absorption in preterm infants fed pooled human milk. *J Pediatr Gastroenterol Nutr* 1986;**5**:638–42.

[20] Schanler RJ, Abrams SA, Garza C. Bioavailability of calcium and phosphorus in human milk fortifiers and formula for very low birthweight infants. *J Pediatr* **113**:95–100.

[21] Greer FR, Tsang RC. Calcium, phosphorus, magnesium and vitamin D requirements for the preterm infant. In: Tsang RC, ed. *Vitamin and mineral requirements in preterm infants.* New York: Marcel Dekker, 1985:99–136.

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Current topic

Diagnosis and management of inborn errors of metabolism

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Few paediatricians will have avoided the predicament of being faced with a desperately ill neonate for whom a diagnosis is not immediately obvious. The majority of such infants will have comparatively common neonatal problems such as systemic infection, congenital heart disease, necrotising enterocolitis, or intracranial haemorrhage. An appreciable minority, however, will have a potentially treatable inborn error of metabolism, and rapid diagnosis and institution of appropriate treatment is essential if a good outcome is to be achieved.

Metabolic disease can present in several ways in the newborn period,[1] but this review will concentrate on those that present acutely and result in death or severe brain damage if not detected and treated.

Before discussing individual diseases, a number of general points must be made. Inborn errors of metabolism are rare and experience is necessary if diagnosis and treatment are to proceed rapidly. As well as expert laboratory and dietetic services, skilled interpretation by a clinician is essential and this will generally lead to transfer to a specialised unit of infants suspected of having one of these diseases. Facilities for initial metabolic investigations must be readily available in each region, and clinicians should be encouraged to use them freely. More specialised investigations—for example, assays of specific enzymes—are needed in only a small number of supraregional laboratories.

Diagnosis and treatment should be aggressive. Some paediatricians are concerned by this therapeutic approach and feel there is a risk of keeping alive a grossly handicapped infant who would otherwise have died peacefully. In practice this is not a problem as it is usually possible to assess prognosis accurately after a short period of energetic treatment. If there is no prospect of reasonably normal cerebral function one can refrain from active measures during the next acute exacerbation. In this way treatment can be assessed at regular intervals, and initially most survivors will be mentally normal or have only minor degrees of brain damage. The long term prognosis for many of the diseases remains guarded, however, even in units providing the best care. The appendix gives some examples of disorders producing severe neonatal illness; more comprehensive lists are available.[1-4]

Clues to the diagnosis of metabolic disease in the newborn

The diagnosis of a metabolic disorder in a seriously ill neonate depends largely on the awareness of the clinicians, but a number of clues can be obtained from the history, clinical examination, and simple 'bedside' tests.

CLUES FROM THE HISTORY

A history of consanguinity is important as many of the disorders are recessively inherited. In addition a previous history of stillbirth or neonatal death in the family is relevant. Ornithine carbamyl transferase deficiency is X linked, and there may be a history of male deaths on the maternal side of the family.

Most affected infants are delivered at or near full term in good condition with a normal birth weight and remain well in the early hours or days of life. The placenta effectively 'haemodialyses' the fetus by removing toxic metabolites, and the fetus has not had contact with substances in the diet (for example, protein) that can precipitate clinical symptoms.

Intense catabolism during the first few days of life together with the introduciton of feeds containing protein unmask the metabolic lesion. Further problems such as starvation, surgery, or superadded infection may all add to the catabolic stress and lead to an exacerbation of symptoms.

A change of feeds may be of importance in an infant with galactosaemia or hereditary fructose intolerance, and a full dietary history is essential. Often the infant will improve when feeds are discontinued only to relapse when milk is restarted, and this episodic pattern of improvement with deterioration should immediately indicate a possible diagnosis.

CLUES FROM THE EXAMINATION

On most occasions physical examination of the affected infant will not lead to a diagnosis. Rarely the typical odour of isovaleric acidaemia, glutaric aciduria type II, or maple syrup urine disease will be noted. Infants with galactosaemia develop cataracts within the first week of life, but otherwise signs and symptoms are non-specific and usually one is faced with a neurologically abnormal, convulsing infant who rapidly proceeds to a comatose state.

CLUES FROM 'BEDSIDE' TESTS

All severely ill neonates will have rapid biochemical and haematological tests carried out. These generally include a full blood count and blood film, estimations of bilirubin, electrolyte and blood glucose concentrations, and acid-base studies. A true metabolic acidosis can easily be missed unless acid-base testing is carried out promptly. A mixed metabolic and respiratory acidosis is common in a severely ill neonate, and the metabolic component may be further masked by mechanical ventilation.

In suspected metabolic disease emphasis has been placed on 'spot' testing of the urine for various compounds. These tests have many shortcomings, are of questionable value, and should not be relied upon to make or refute a diagnosis—especially if specific metabolic screening methods are available in all regions. Urine testing for reducing substances is still often mentioned by referring clinicians who suspect galactosaemia. We have seen a number of neonates with galactosaemia whose urine did not show any reducing substances, despite their being severely ill. In others severe renal tubular disease limits the value of urine testing. A specific screening test for classical galactosaemia must be carried out as a matter of urgency on all infants suspected of having the disease, and this test must be readily available in all regions.

A urine test for ketones can be useful. Heavy ketonuria is unusual in the neonatal period and its detection should be immediately followed by organic acid analysis.

In patients with acidosis, calculation of the anion gap—the sum of the serum concentrations of sodium and potassium minus the sum of the serum concentrations of chloride and bicarbonate (mmol/l)—can be helpful. Patients with an increased anion gap, and especially those with a value greater than 25 mmol/l (normal 12–16 mmol/l), are likely to have a specific organic acidaemia. Patients with a normal anion gap and acidosis are most likely to have renal tubular acidosis or intestinal bicarbonate loss.[5 6]

The metabolic screen

Metabolic tests must be carried out urgently, at the same time as tests to detect infections and other common neonatal illnesses. One runs the risk of missing the diagnosis if a delay is caused while the results of an infection screen or echocardiogram are awaited before considering metabolic investigations. Occasionally we are asked to give advice after an infant's death, and although we are able to perform some metabolic investigations (see below) early suspicion and collection of the appropriate tissues and fluid from a living child permits a more comprehensive screen for metabolic disease.[7 8]

Although techniques vary, most laboratories will require a sample of blood (1–2 ml in a heparinised tube) and urine (5–10 ml in a sterile container with no preservatives) for a metabolic screen. If an urgent result is required it is essential that the referring clinician contacts the laboratory in advance to indicate the urgency. Full details of diet and drugs must be included, and the samples should be transported rapidly to the laboratory. Where possible blood must be taken before any blood transfusion, as this will interfere with the screening test most laboratories use to exclude galactosaemia.[9] If this is not possible the laboratory personnel must be told about the transfusion so that an alternative test can be used. It is good clinical practice to save a sample of blood from all infants who require exchange transfusion in case screening for galactosaemia is later thought to be necessary.

In our laboratory amino acids concentrations are estimated in blood and urine by one dimensional paper chromatography. Abnormal results are then characterised quantitatively. Gas liquid chromatography with mass spectrometry is used for organic acid analyses. The plasma ammonia concentration should be measured, and a portion of the urine specimen kept for orotic acid estimation if the ammonia concentration is high. Occasionally more specialised investigations will be required—for example, amino acid or organic acid analysis on cerebrospinal fluid—and if this is the case it is best to discuss the clinical problem with the laboratory to ensure that the appropriate samples are collected.

Infants with lactic acidosis present a difficult problem. It is difficult to distinguish primary lactic acidosis caused by a defect in pyruvate metabolism from secondary lactic acidosis caused by hypoxia, cardiac disease, infection, or convulsions. It is necessary to treat possible underlying causes aggressively, while trying to separate the two groups. Plasma lactate and pyruvate are extremely sensitive to the method of collection, and venous obstruction by crying, torniquet, or restraint can cause a two to three fold increase in concentrations. The urinary lactate concentration is useful for monitoring if blood lactate is greater than 6 mmol/l. In those patients with secondary lactic acidosis, the urinary lactate concentrations fall as the underlying disorder improves, while infants with a primary defect are unresponsive to most treatments. Often the child dies before a clear distinction is made and one has to rely on formal assay of the enzymes known to cause lactic acidoses. This is an unsatisfactory approach as many patients with strong evidence of a primary defect have no biochemical abnormality on testing. Lactic acidosis is a heterogenous group of disorders and it is possible that many defects have not been fully defined.[10] With other disorders we would expect to confirm the diagnosis of a urea cycle defect, amino acid disorder, or organic acidaemia within six hours of receiving the samples (and often sooner).

Management while awaiting results

For most infants with severe symptoms in the newborn period active treatment is required, and at this point consideration of transfer to a specialised unit should be made. General measures will include correcting electrolyte imbalance and treating acidosis with sodium bicarbonate; often large doses of bicarbonate are necessary and we prefer to give it in a steady intravenous infusion over 24 hours. There is always a risk of inducing hypernatraemia, but with acidosis so resistant to treatment dialysis should be considered early. Mechanical ventilation is usually required for severely affected infants.

The key to treatment is to try and induce an anabolic state as quickly as possible. A high energy intake, using 15–20% dextrose infusions together with insulin (1U/3 g glucose, or approximately 0·05 U/kg/hour) and lipid solutions may help to initiate anabolism. It is prudent to insert a central venous line as this regimen rapidly damages peripheral veins and adequate venous access is of vital importance. Though this regimen is suitable for most disorders, patients with a primary lactic acidosis may be made worse by a carbohydrate load and for these patients the next phase of treatment (removal of toxic metabolites) becomes of paramount importance. A number of studies have compared the efficacy of exchange transfusion, haemodialysis, and peritoneal dialysis in the acute management of inborn errors.[11] We prefer to use peritoneal dialysis, withholding exchange transfusion for the rare case in which immediate access to peritoneal dialysis is not possible. The beneficial effects of exchange transfusion are transient. In most infants with organic acid disorders, dialysis is likely to be needed for at least seven days and once established we introduce protein cautiously, starting at 0·5 g/kg/day and increasing daily as tolerated, up to 1·2–1·5 g/kg/day. In addition, at least 0·502 MJ/kg/day are supplied and this combination is usually sufficient to achieve anabolism and promote growth.

Children with hyperammonaemia respond rapidly to treatment with sodium benzoate, ammonia nitrogen being diverted away from the defective urea cycle by being conjugated with glycine to form hippurate. Sodium benzoate should be used in conjunction with dialysis in those infants with hyperammonaemia of greater than 600 μmol/l, or alone in those infants with a more moderate increase. The effect is transient, and a loading dose of 250 mg/kg should be followed by a constant infusion of 250 mg/kg/day, depending on response. In experimental animals sodium benzoate potentiates ammonia toxicity, although this may be prevented by giving L-carnitine first. We have not noted any adverse effect on our patients.[12 13]

The megavitamin 'cocktail'

Several inborn errors of metabolism have vitamin responsive forms, and often a combination of vitamins in pharmacological doses are given intravenously to sick infants while awaiting results. A typical 'cocktail' is shown in the table. It is important that this therapeutic approach be considered only after the appropriate specimens have been collected for metabolic investigations. It is reasonable to try the 'cocktail' in infants in whom no diagnosis has been established, but in our experience this approach is rarely effective. When a diagnosis is made of an inborn error known to have a vitamin

Table *Megavitamin 'cocktail'*

Ingredient	*Mg/day*
Vitamin B_{12}	1·0
Biotin	100
Thiamine	50
Riboflavine	50
Nicotinamide	600
Pyridoxine	100

responsive form, that vitamin should be given in a dose about 100 times the daily requirement until the cofactor responsiveness has been assessed.[14]

Specific management after diagnosis

UREA CYCLE DEFECTS

After dialysis has been used to reduce plasma ammonia concentrations rapidly, protein restriction with supplementary essential amino acids forms the basis of treatment. Long term oral sodium benzoate is often necessary and, in all the disorders except arginase deficiency, arginine supplementation becomes essential if chronic hyperammonaemia is to be avoided.[15-17]

ORGANIC ACID DISORDERS

Acute management includes dialysis and protein restriction. In addition there is a direct toxic effect on the tissues from a build up of acyl coenzyme A esters within the cell as a result of the metabolic block. Carnitine plays an important part by conjugating with these compounds to form acylcarnitine esters, which are excreted through the kidney. This leads to a secondary carnitine deficiency and has led to the use of carnitine supplementation in both the acute and chronic management of organic acidaemia.[18]

In isovaleric acidaemia, isovaleryl coenzyme A accumulates and the isovaleryl groups can effectively be conjugated to glycine. Glycine supplementation can therefore increase the excretion of isovaleryl glycine leading to metabolic correction.[19]

Antimicrobials (for example, metronidazole) have been used to sterilise the gut in an attempt to reduce substrate production from gut organisms in some organic acid disorders.[20]

Management when death seems inevitable or the child dies despite treatment

Even if no diagnosis is established, aggressive treatment is justified as some defects are transient—for example, transient hyperammonaemia of prematurity. If death seems inevitable it is important to gather as much information about the child as possible. Necropsy is essential and careful thought is needed to ensure that the correct samples are collected. The necropsy must be carried out as soon as possible after death so that biochemical studies can be made on tissues that have not yet undergone enzymatic self digestion. The need for necropsy should be discussed with the parents before death and permission obtained at this time. Indeed, many couples find it easier to discuss this matter before rather than after death. If possible before death 1 ml of serum or plasma should be collected and frozen together with as much urine as can be collected (even a few drops can be useful). If metabolic investigations are considered for the first time after death one can try and retrieve samples, taken during life, from routine pathology laboratories. Blood from cardiac puncture can be collected, separated, and stored. Cerebrospinal fluid is usually easy to obtain after death by cisternal puncture and can be used for amino acid and organic acid analyses. It is essential to set up a fibroblast culture, as many enzyme assays can be performed if an inborn error is suggested by necropsy findings. The results of the enzyme tests are not affected by enzymatic self digestion and, in addition, the fibroblasts can be used as a source of DNA. If permission to carry out a full necropsy is not obtained (perhaps for religious reasons) it is important to ask the parents to agree to limited biopsy study—for example, liver and muscle. Many (but not all) enzymes can be accurately assayed many hours after death, and even if more than 12 hours has elapsed it is still worth collecting samples for measurement of metabolites resistant to rapid change after death—for example, for diagnosis of fatty acid oxidation defects. Biopsy samples should be shared with the pathologist who will require material for essential histology, histochemistry, and electron microscopy. Samples for biochemical studies should be snap frozen (in dry ice or liquid nitrogen) and stored at −70°C until assayed.[21]

Conclusions

Inborn errors of metabolism are an important cause of neonatal morbidity and mortality. In many infants they are not diagnosed, and the children die without the benefit of available treatment. In addition, the parents of these children do not receive the appropriate genetic advice and often have the additional problems of further affected infants before the underlying disorder is detected.

It is important to consider an inborn error as a potential cause of any severe neonatal illness and to have a systematic approach to diagnosis and management.

Each region should have facilities to screen the sick newborn rapidly for metabolic disease, as well as a clinician whose main interest is in the management of inborn errors of metabolism.

Appendix *Metabolic disorders that may present as severe neonatal illness*

● Carbohydrate metabolism

Galactosaemia produces cataracts and severe liver disease; the other members of this group usually present with a combination of hypoglycaemia and lactic acidosis. Hereditary fructose intolerance requires a fructose load before symptoms appear.

Disorders	*Enzyme deficiencies*
1 Galactosaemia ('classical')	Galactose-1-phosphate uridyl transferase
Epimerase	Uridine diphosphate-galactose-4-epimerase
2 Glycogen storage disease	
Type Ia	Glucose-6-phosphatase
Type Ib	'Translocase'
3 Fructose	Fructose-1-6-diphosphatase Fructose-1-phosphate aldolase (Hereditary fructose intolerance)
4 Lactic acidosis	Pyruvate carboxylase Pyruvate dehydrogenase Phosphoenolpyruvate carboxykinase

● Amino acid disorders

Presentation may be variable, but central nervous system dysfunction is common. In maple syrup urine disease the urine has a characteristic odour. Acute hereditary tyrosinaemia causes severe liver disease and renal tubular dysfunction.

Disorders	*Enzyme deficiencies*
5 Maple syrup urine disease	Branched chain ketoacid decarboxylase
6 Non-ketotic hyperglycinaemia	Glycine cleavage system
7 Tyrosinaemia type I	Fumryl acetoacetase

● Organic acid defects

Present with a combination of lethargy, seizures, ketoacidosis, neutropenia, hyperammonaemia, and hyperglycinaemia. Hypoglycaemia is common. Isovaleric acidaemia and glutaric aciduria type II produce a specific odour of 'sweaty feet'.

Disoders	*Enzyme deficiencies*
8 Methylmalonic acidaemia	Methylmalonyl coenzyme A mutase (and others)
9 Propionic acidaemia	Propionyl coenzyme A carboxylase
10 Isovaleric acidaemia	Isovaleryl coenzyme A dehydrogenase
11 Glutaric aciduria type II	Glutaryl coenzyme A dehydrogenase
12 Dicarboxylic aciduria	Various acyl coenzyme A dehydrogenases

● Urea cycle defects and hyperammonaemia

Disorders	*Enzyme deficiencies*
13 Carbamyl phosphate synthetase deficiency	
14 Ornithine carbamyl transferase deficiency	
15 Citrullinaemia	Argininosuccinic acid synthetase
16 Argininosuccinic aciduria	Argininosuccinic acid lyase
17 Argininaemia	Arginase
18 Transient hyperammonaemia of prematurity	Unknown

● Miscellaneous

Disorders	*Enzyme deficiencies*
19 Pyridoxine dependent seizures	Unknown
20 Lysosomal storage disease*	Various
21 Peroxisomal disorders†	Various

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*A number of lysosomal storage disorders have been reported as presenting with hydrops fetalis.[3] †Can produce profound neurological dysfunction in the newborn period.[4]

References

[1] Burton BK. Inborn errors of metabolism: the clinical diagnosis in early infancy. *Pediatrics* 1987;**79:**359–69.

[2] Danks DM, Wraith JE, Brown GK. Inborn errors—diagnosis and management. In: Levene M, Bennett MJ, Punt J, eds. *Fetal and neonatal neurology and neurosurgery*. Edinburgh: Churchill Livingstone, 1988:459–68.

[3] Gillan JE, Lowden JA, Gaskin K, Cutz E. Congenital ascites as a presenting sign of lysosomal storage disease. *J Pediatr* 1984;**102:**225–31.

[4] Stephenson JBP. Inherited peroxisomal disorders involving the nervous system. *Arch Dis Child* 1988;**63:**767–70.

[5] Oh MS, Caroll HJ. The anion gap. *N Engl J Med* 1977;**297:** 814–7.

[6] Gabow PA, Kaehny WD, Fennessey PV, Goodman SI, Gross PA, Schrier RW. Diagnostic importance of an increased anion gap. *N Engl J Med* 1980;**303:**854–8.

[7] Chalmers RA, Watts RWE, Lawson AM. A comprehensive screening method for detecting organic acidurias and other metabolic diseases in acutely sick infants and children. *Ann Clin Biochem* 1977;**14:**149–56.

[8] Holton JB. Diagnosis of inherited metabolic diseases in severely ill children. *Ann Clin Biochem* 1982;**19:**389–95.

[9] Beutler E, Baluda MC. A single spot test for galactosaemia. *J Lab Clin Med* 1966;**681:**137–41.

[10] Robinson BH, Taylor J, Sherwood WG. The genetic heterogeneity of lactic acidosis: occurrence of recognisable inborn errors of metabolism in a pediatric population with lactic acidosis. *Pediatr Res* 1980;**14:**956–62.

[11] Saudubray JM, Ogier H, Charpentier C, *et al.* Neonatal management of organic aciduria. Clinical update. *J Inherited Metab Dis* 1984;**7**(suppl 1):2–9.

[12] O'Connor JE, Ribelles M, Grisolia S. Potentiation of hyperammonaemia by sodium benzoate in animals. A note of caution. *Eur J Pediatr* 1982;**138:**186–7.

[13] O'Connor JE, Costrell M, Grisolia S. The potentiation of ammonia toxicity by sodium benzoate is prevented by L-carnitine. *Biochem Biophys Res Commun* 1987;**145:**817–24.

[14] Leonard JV, Daish P. Evaluation of cofactor responsiveness. *J Inherited Metab Dis* 1985;**8**(suppl 1):17–9.

[15] Batshaw ML, Brusilow S, Waber L, *et al.* Treatment of inborn errors of urea synthesis. Activation of alternative pathways of waste nitrogen synthesis and excretion. *N Engl J Med* 1982;**306:** 1387–92.

[16] Brusilow S. Arginine, an indispensible amino acid for patients with inborn errors of urea synthesis. *J Clin Invest* 1984;**74:** 2144–8.

[17] Wendell U, Wieland J, Bremer HJ, Bachmann C. Ornithine transcarbamylase deficiency in a male: strict correlation between metabolic control and plasma arginine concentration. *Eur J Pediatr* 1989;**148:**349–52.

[18] Stanley CA. New genetic defects in mitochondrial fatty acid oxidation and carnitine deficiency. *Adv Pediatr* 1987;**34:**59–88.

[19] Naglak M, Salvo R, Madsen K, Dembure P, Elsas L. Treatment of isovaleric acidemia with glycine supplement. *Pediatr Res* 1988;**24:**9–13.

[20] Bain M, Borriello SP, Reed PJ, *et al.* Therapeutic potential of antibiotics in methylmalonic acidaemia. *Pediatr Res* 1985;**19:** 1083.

[21] Kronick JB, Scriver CR, Goodyer PR, Kaplan PB. A perimortem protocol for suspected genetic disease. *Pediatrics* 1983;**71:**960–3.

Correspondence to Dr JE Wraith, Willink Biochemical Genetics Unit, Royal Manchester Children's Hospital, Manchester M27 1HA.

Archives of Disease in Childhood, 1989, **64,** 1416–1417

Perinatal lessons from the past

Dr John Burns (1774–1850) and neonatal jaundice

P M DUNN

University of Bristol, Southmead Hospital, Bristol

John Burns, the son of a minister, qualified in medicine in Glasgow. Becoming a surgeon's clerk at the Royal Infirmary on its opening in 1792, he studied and lectured on anatomy and midwifery, and published his first work *The Anatomy of the Gravid Uterus* in 1799 when he was 25. The following year he was appointed surgeon to the Royal Infirmary and in 1815 succeeded to the Chair of Surgery in the University of Glasgow. He was a most popular teacher, his lectures often being attended by 200 students, and he acquired a large private practice. Of the several books he wrote, his main treatise was *The Principles of Midwifery* published in 1809.[1] This work rapidly became famous and was reprinted many times both in the United Kingdom and in America. Like most of the physician-accoucheurs of the 18th and 19th centuries, Burns was a paediatrician as well as an obstetrician and Book IV of his treatise had the title *Of the Management and Diseases of Children.* The text abounds with descriptions and insights that confirm him to have been a man of rare perception and originality. The extract on neonatal jaundice, reprinted below, is remarkable in that it distinguishes between the obstructive and non-obstructive varieties and draws attention to the danger of convulsions and death. Burns also hints at what would now be termed the enterohepatic circulation of bilirubin.

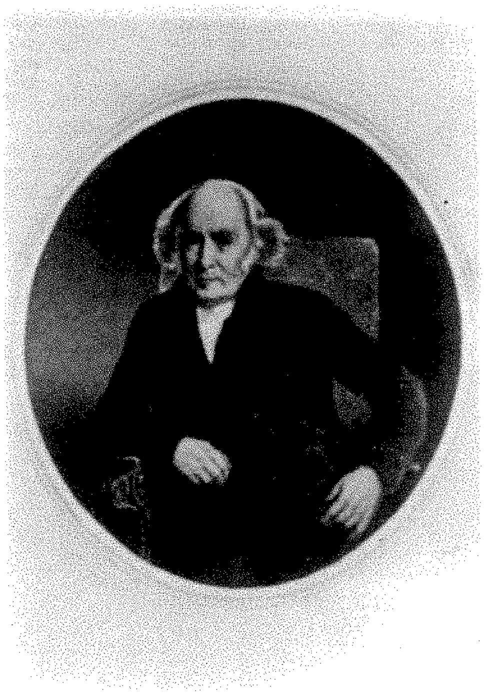

Figure *Professor John Burns of Glasgow, 1774–1850.*

'The jaundice of infants is a disease attended with great danger, especially if it appears very soon after birth, and the stools evince a deficiency of bile; for we have then reason to apprehend some incurable state of the biliary apparatus. I conceive that there are two species of this disease, which are very opposite in their nature. In the first, there is an obstacle to the passage of the bile into the intestine, the child is costive, and the meconium is paler than usual, and after it is removed, the stools become light-coloured; the skin, very early after birth, becomes of a deep yellow colour, which extends to the eyes. The child sucks very little, has occasionally a difficulty in swallowing, is languid, becomes emaciated, moans much, is troubled with flatulence, sometimes with cough and phlegm in the trachea, or vomiting, convulsions, colic, and fever, occasionally supervene. In some cases, the liver is felt enlarged, and the hypochondrium is tumid. The water is very high-coloured. This disease often proves fatal in a week, but it has been known to continue in variable degrees of violencé for a considerable time, and at last to disappear, though such children continue long delicate. With regard to the cause of this disease, we find, that sometimes it consists of obstruction of the hepatic duct or ductus communis, either by thickening of the coats, or pressure, in consequence of enlargement of some part in the vicinity of the duct; or it may consist in imperforation of the duct. Sometimes it proceeds from temporary obstruction in the duct, owing to viscidity of the bile. Now some of these cases are irremoveable, others are not; but as we cannot *a priori* say what the cause may be, in any particular instance, we must use the means of cure in every case. The most likely remedies for removing this disease, are gentle emetics, given very early and followed by the exhibition of half a grain of calomel, morning and evening, till the bowels are acted on; or we may give this medicine even three times a-day, in some cases; but we must be cautious not to induce much purging, or push the mercury far, lest we bring on fits.

The second species differs from the first, in the stools being dark-coloured or green, showing that there is no obstruction, or at least no permanent obstruction, to the passage of the bile. Like the first species it appears soon after birth, and is accompanied with great oppression, moaning, colic, and convulsive affections. It is attended with much danger, and frequently carries off the infant in a few days. The early use of calomel would appear to be the most proper practice, and the strength must be supported in all those cases by the breast milk, given with the spoon, if the child won't suck, and small doses of white wine whey.

Jaundice, appearing at a considerable period after birth, does not require a separate consideration here, nor is it a very common occurrence.'

Professor Burns was deeply involved with parliamentary and other business connected with the university. In 1850 he was drowned when the steamer *Orion*, on which he was returning to Glasgow, was wrecked near Portpatrick. He was 76.

Reference

[1] Burns J. *The principles of midwifery; including the diseases of women and children*. London: Longman, 1817:602.

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Annotation

Salmonellas and eggs

Epidemiology

Salmonella food poisoning is a common cause of human illness in the United Kingdom, and in the last two years the incidence has increased appreciably. Much of the increase can be attributed to a particular subtype of one of the many serotypes, *Salmonella enteritidis* phage type 4 (PT4). This is predominantly a poultry serotype, but the current epidemic has been attributed not only to poultry meat but also to a more unusual source, hens' eggs.[1] Furthermore *S enteritidis* PT4 is not the only strain that has recently been responsible for egg associated outbreaks. Other phage types of *S enteritidis* have caused egg associated outbreaks in hotels in 1988 (Communicable Disease Surveillance Centre (CDSC), unpublished observations), and *S typhimurium* PT49 was responsible for a widely publicised incident associated with eggs used to make mayonnaise at the House of Lords.[2]

The number of cases of human salmonellosis reported to the Public Health Laboratory Service (PHLS) CDSC in 1978 was 9042, and only 661 (7·3%) of these were *S enteritidis*. In 1987, 16 991 cases of salmonella were reported and 5653 (33·3%) of these were *S enteritidis* (CDSC, unpublished observations) and for 1988, the provisional estimates are that 12 500 of 23 000 salmonella infections (54·3%) were *S enteritidis*. At present, about 35% of the *S enteritidis* isolates reported are in those under 15 years of age. Although children and infants are more likely than older people to have stool samples cultured, there is undoubtedly considerable underascertainment of salmonella infection in all age groups.[1]

Salmonella infection can lead to serious complications especially in children. In the 10 years to 1987, 1655 salmonella bacteraemias were reported to the PHLS CDSC, and of these 312 (19%) were in patients under 15 years old, and 124 (7%) in those under 1 year (CDSC, unpublished observations). The corresponding figures for salmonella meningitis were 40 in total, 31 (78%) in patients under 15 years old and 27 (68%) in those under 1 year old. Although the proportion of salmonella infections progressing to bacteraemia does not appear to be increasing, the total annual number of salmonella bacteraemia has risen from 131 to 226 during this 10 year period.

Causes

Outside the hospital setting, where person to person spread of salmonella occurs more commonly than in the community, the main risk factor in the acquisition of salmonella infection is consumption of contaminated food. In the unweaned child, contaminated milk is clearly the most likely source, and community outbreaks have been caused by dried infant milk formulas,[3] by unpasteurised cows' milk, and by failed pasteurisation of bottled milk.[4] In the weaned and older child, the popularity of egg and egg based foods is considerable, and the widespread contamination of eggs poses a serious problem. Children have been particularly affected in other recent foodborne salmonella outbreaks including outbreaks caused by *S napoli* in contaminated chocolate[5] and *S typhimurium* in salami sticks.[6] These outbreaks were curtailed, however, by the simple expedient of withdrawing the branded food product until the manufacturing processes were modified. With eggs, however, where the daily consumption is 30 million eggs a day from 45 000 producers, the nature and size of the problem are on another scale and a different approach is required.

Prevention

Although salmonellas of certain serotypes have long been known to be capable of infecting hens' ovaries and their egg contents[7] these serotypes rarely cause human illness. The fact that the strain now most commonly associated with human illness is capable of contaminating the inside of an egg[1] means that merely adhering to the simple hygienic practices that prevent the contamination of the egg contents from the shell is no longer adequate.

A simple but effective policy would be to recommend avoidance of all raw or lightly cooked egg dishes. In those for whom any risk is unacceptable, this is indeed the only solution. In formulating guidance it is important to consider the consumer, and the fact that eggs are a nourishing, cheap, and convenient food. In infants and children the no risk policy is advisable. Eggs should be boiled for at least six minutes and can be mashed or liquidised with milk for infants who are being weaned. In the healthy child, the probability of a single lightly boiled, scrambled, or fried egg causing a serious salmonella infection is very small,[1] but a mother who provides this type of food on one occasion is likely to repeat the hazard daily. Any procedure that increases the chances of using an infected egg—such as mixing a large number of fresh eggs—should be avoided, so that consumption of portions of scrambled eggs or omelettes made in bulk for catering purposes is inadvisable. The elementary rules of food hygiene should be strictly followed with all egg dishes. This means that the egg should be stored for as short a time as possible, it should be stored in a refrigerator, then allowed a few minutes to reach room temperature before cooking, and eaten immediately after cooking. Raw eggs and all dishes containing raw eggs should be avoided altogether, and this advice has the advantage of being somewhat easier to follow. Liquid pasteurised egg is occasionally available in domestic sized cartons from supermarkets, and its use should be considered if recipes specify raw egg.

However careful the consumer is, control of the epidemic of *S enteritidis* PT4 in babies, children, and adults requires the eradication of the organism from layer and broiler chicken flocks, and so is ultimately dependent upon the work of veterinarians and the poultry industry.

References

[1] Public Health Laboratory Service. Memorandum of evidence to the Agriculture Committee Enquiry on salmonella in eggs. *Public Health Laboratory Service Microbiology Digest* 1989;**6:** 1–9.

[2] Mitchell E, O'Mahony M, Lynch D, *et al.* Large outbreaks of food poisoning caused by Salmonella typhimurium definitive type 49 in mayonnaise. *Br Med J* 1989;**298:**99–101.

[3] Rowe B, Begg NT, Hutchinson DN, *et al.* Salmonella ealing infections associated with consumption of infant dried milk. *Lancet* 1987;**ii:**900–3.

[4] Barrett NJ. Milkborne disease in England and Wales in the 1980s. *Journal of the Society of Dairy Technology* 1989;**42:**4–6.

[5] Gill ON, Bartlett CLR, Sockett PN, *et al.* Outbreak of Salmonella napoli infection caused by contaminated chocolate bars. *Lancet* 1983;**i:**574–7.

[6] Cowden JM, O'Mahony M, Bartlett CLR, *et al.* A national outbreak of Salmonella typhimurium DT 124 caused by contaminated salami sticks. *Epidemiology and Infection* (in press).

[7] Hooper SA, Mawer SL. Salmonella enteritidis in poultry. *Veterinary Record* 1988:**123:**351.

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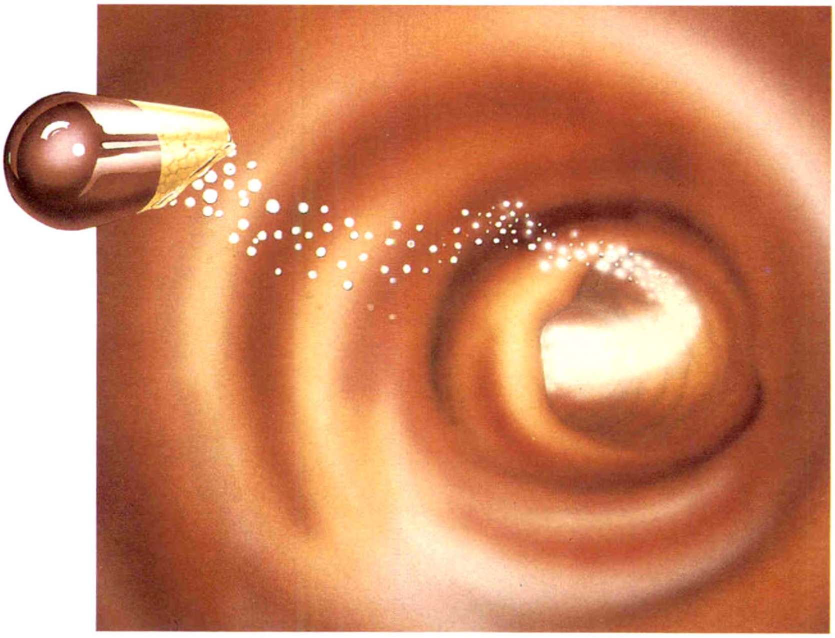

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Diagnostic accuracy of pH monitoring in gastro-oesophageal reflux

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SUMMARY One hundred and eleven children admitted with suspected gastro-oesophageal reflux were studied, with 24 hour oesophageal pH monitoring as the first line of investigation. Barium swallow examination, or oesophagoscopy, or both, were carried out only in children with abnormal pH, who subsequently had a trial of 1–12 months medical treatment. All patients were followed up for eight months to two years. A final diagnosis of gastro-oesophageal reflux was made in 41 patients, in all of whom the pH study was abnormal (100% sensitivity). The final diagnosis was different in 70 patients; 66 of these had a normal pH (94% specificity). All children with gastro-oesophageal reflux were treated with drugs. All those with a percentage reflux time of more than 27 and more than 20 episodes of reflux lasting more than 5 minutes failed to improve and needed operation.

We conclude that monitoring of the oesophageal pH should be the first line of investigation in patients with gastro-oesophageal reflux and should be used together with clinical data and other investigations, to identify those children who will need operation.

Early diagnosis of gastro-oesophageal reflux is important for the prompt institution of antireflux treatment and the prevention of potentially serious complications—for example, severe oesophagitis, oesophageal stenosis, and aspiration syndrome.

Several methods for the diagnosis of gastro-oesophageal reflux are available. Among them 24 hour oesophageal pH monitoring is considered the most sensitive and the most specific.[1 2] Nevertheless it is not yet common practice to use this as the initial investigation for patients with suspected gastro-oesophageal reflux. There is a tendency to prefer traditional tests like barium swallow,[3-5] which are considered to be more practical.

In addition, no investigation seems able to predict those patients who will eventually need operation. In fact it is still widely held that the decision to recommend operation should be based upon the clinical history and failure of drug treatment.[3-8]

We have therefore evaluated 24 hour oesophageal pH monitoring as the first line of investigation in the diagnosis of gastro-oesophageal reflux to determine whether predictions can be made based solely on this investigation as to which patients will require surgical and which will require medical treatment.

Patients and methods

From January 1985 to December 1987, 111 infants and children aged 1 month to 13·5 years (median 9·3 months) were admitted with signs and symptoms typical of gastro-oesophageal reflux. The presenting complaints are listed in table 1.

Twenty four hour oesophageal pH monitoring was routinely carried out in all patients with a 3 mm diameter combined glass probe (pH electrode plus

Table 1 *Presenting symptoms in the 111 infants and children admitted for suspected gastro-oesophageal reflux*

	No (%) of infants
Vomiting	69 (62)
Failure to thrive	29 (26)
Feeding difficulties	20 (18)
Haematemesis	15 (13)
Recurrent pneumonia	14 (12)
Recurrent wheezing	12 (11)
Chronic cough	11 (10)
Apnoeic spells	11 (10)
Iron deficiency anaemia	10 (9)
Epigastric pain	10 (9)
Irritability	7 (6)

reference electrode: lot 440/M3 Ingold), connected to a computerised pH recorder (Proxima 1–2 Exor). This instrument allows the collection of data about oesophageal pH by sampling, which can be programmed for frequency and duration. We use the 24 hour pH monitoring with sampling every eight seconds.

The probe was calibrated in pH 4 and pH 7 buffer solutions before and after use. It was introduced into the stomach transnasally and then retracted and placed above the lower oesophageal sphincter at a point equal to 13% of the distance between the nares and the lower oesophageal sphincter calculated by the formula of Strobel *et al.*[9]

During the test, children were allowed unrestricted diet and activity. The instrument is battery powered, small and light weight. It is carried in a leather case fitted with a shoulder strap so the patients are able to enjoy considerable freedom of movement.

The data are memorised on a solid state memory and are rapidly analysed and printed out: the instrument prints the pH graph as a function of time, and also records the following measurements: (i) total percentage reflux time; (ii) total number of episodes of reflux in 24 hours; (iii) number of episodes of reflux lasting more than five minutes in 24 hours; (iv) duration (in minutes) of the longest episode of reflux; (v) the mean duration (in minutes) of all the reflux episodes.

An episode of reflux is defined as a drop in the oesophageal pH to a value of 4 or less for at least eight seconds.

Normal values (table 2) have been obtained from 14 asymptomatic infants and children aged from 3 to 68 months (median 12·5 months), who had no history or symptoms suggestive of gastro-oesophageal reflux. The upper limit of normal for all measurements was arbitrarily defined as two standard deviations above the mean value of each value measured in the control subjects.

In patients with a normal oesophageal pH tracing no further investigations were carried out and no treatment was instituted, but regular follow up was arranged for a period of time ranging from eight months to two years.

A barium swallow examination or oesophagoscopy, or both, were carried out only in children whose oesophageal pH was abnormal. This group of patients then underwent a one to 12 months' trial of medical treatment, which consisted of a combination of thickened feeding, sleeping in the prone position with their shoulders raised by 30°, bethanechol and, for documented oesophagitis, antacids, and ranitidine. Only patients in

Table 2 *Mean (SD) normal values of 24 hour pH monitoring in control group (n=14)*

Percentage reflux time	2·08 (1·75)
No of episodes of reflux	23·71 (20·70)
No of episodes of reflux lasting >5 minutes	0·85 (0·86)
Duration of the longest episode of reflux (min)	7 (4·37)
Mean duration of all episodes of reflux (min)	1·33 (0·52)

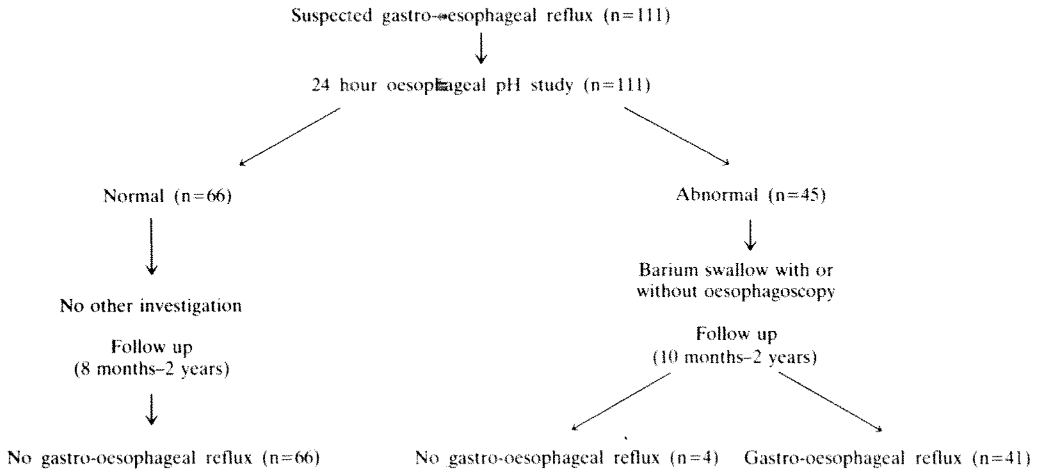
Suspected gastro-oesophageal reflux (n=111)
24 hour oesophageal pH study (n=111)
Normal (n=66)
Abnormal (n=45)
No other investigation
Follow up
(8 months–2 years)
Barium swallow with or
without oesophagoscopy
Follow up
(10 months–2 years)
No gastro-oesophageal reflux (n=66)
No gastro-oesophageal reflux (n=4)
Gastro-oesophageal reflux (n=41)

Fig 1 *Diagnostic flow chart.*

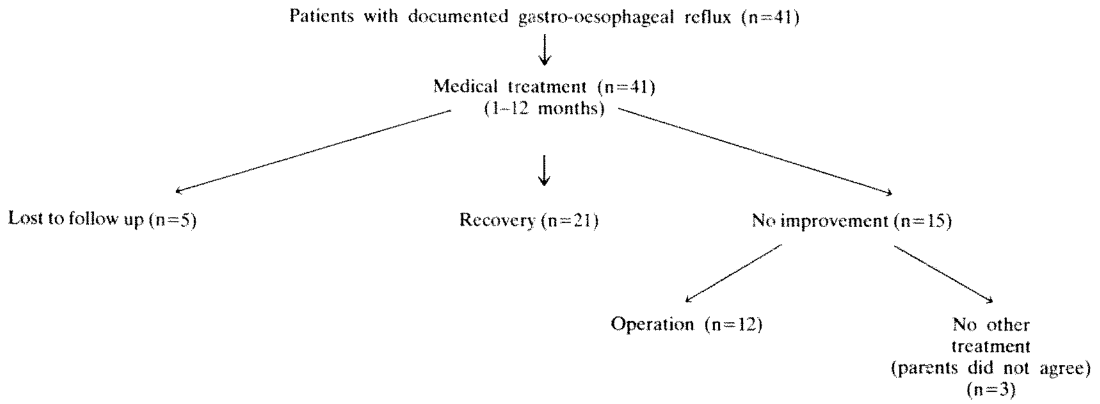

Fig 2 *Therapeutic flow chart for 41 patients with gastro-oesophageal reflux.*

whom medical treatment had failed were offered operation.

The sensitivity, specificity, and positive predictive value of 24 hour pH monitoring for the detection of gastro-oesophageal reflux were calculated.

Results from those patients who responded to medical treatment and those who required operation were compared by the Student's t test.

Results

The diagnostic procedures and the first results are summarised in fig 1.

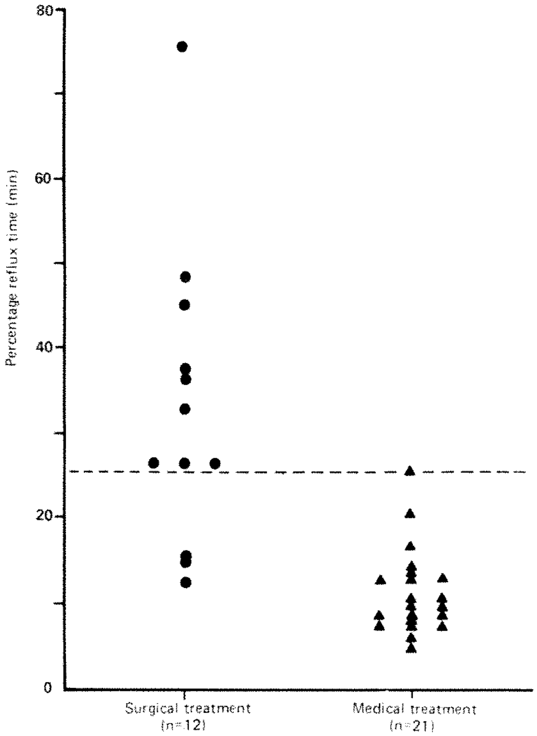

Fig 3 *Percentage of reflux time in the two groups. Mean and three standard deviations is shown for group receiving medical treatment (-----).*

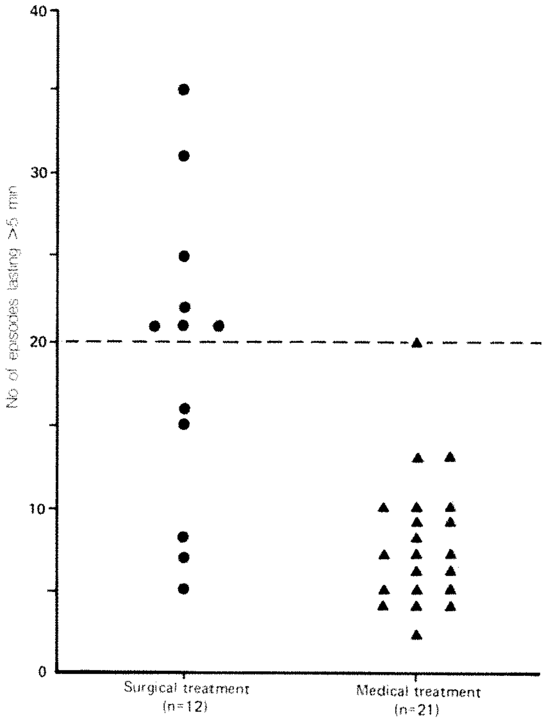

Fig 4 *No of episodes of reflux lasting longer than 5 minutes in the two groups. Mean and three standard deviations is shown for group receiving medical treatment (-----).*

Table 3 *Comparison of the mean (SD) reflux values between surgically and medically treated patients*

	Surgical treatment (n=12)	*Medical treatment (n=21)*	*p Value**
Percentage reflux time	33·46 (16·88)	11·69 (4·98)	0·001
No of episodes of reflux	157·75 (59·76)	80·57 (61·42)	0·002
No of episodes of reflux lasting >5 minutes	19·75 (9·47)	7·81 (3·96)	0·001
Duration of longest episode of reflux (min)	52·83 (42·76)	28·05 (31·28)	0·1
Mean duration of all episodes of reflux (min)	3·42 (2·39)	2·86 (1·66)	0·5

*Student's *t* test.

Of the 111 pH studies performed, 66 were normal. The diagnosis of gastro-oesophageal reflux in these patients was further ruled out during the clinical follow up.

In the remaining 45 the percentage of reflux time as well as at least two other measurements were abnormal.

In 41 of the 45 children with abnormal pH studies the diagnosis of gastro-oesophageal reflux was confirmed by a barium swallow examination or oesophagoscopy, or both. Four children had different final diagnoses (coeliac disease, parasternal hernia of Morgagni, recurrent urinary tract infections, and megaoesophagus).

The test has 100% sensitivity (41 out of 41) in the diagnosis of gastro-oesophageal reflux and a specificity of 94% (66 out of 70), with a positive predictive value of 91%.

Symptoms disappeared in 21 (51%) of the 41 children suffering from gastro-oesophageal reflux who underwent medical treatment (fig 2), while they persisted in 15 (37%). Of this last group, 12 children underwent operation with complete resolution of symptoms and normalisation of the oesophageal pH.

In three patients who are still suffering from gastro-oesophageal reflux, operation has not yet been carried out as parents have withheld consent. Five children (12%) were lost to follow up.

The success of medical treatment was higher in children under 18 months of age (17 of 23, 74%) compared with those who were older (four of 13, 31%).

Severe neurological damage was present in four children: two did not respond to conservative treatment and required operation.

Table 3 shows the pH results in the medically treated and the surgically treated patients. There were significant differences between the two groups for three measurements: the percentage of total reflux time (p=0·001), the number of episodes lasting longer than five minutes in 24 hours (p=0·001), and the total number of episodes of reflux in 24 hours (p=0·002).

The results of the pH studies in the three children who did not improve after 12 months of medical treatment and who have not yet had operations were similar to those of the surgical group.

On analysing the measurements for each patient we found an overlap for each measurement of the values of the medically and surgically treated groups: the least overlap was seen in the percentage of reflux time and in the number of episodes of reflux lasting longer than five minutes. In 9/12 (75%) and 7/12 (58%) of cases, respectively, these values were higher than both the maximum value and the average value plus three standard deviations of those of the patients successfully treated medically (figs 3 and 4).

Discussion

Some authors routinely carry out a barium swallow examination,[3-5] which is thought to be more useful than pH monitoring despite its poor sensitivity and specificity,[10 11] and others recommend further investigations (oesophagoscopy, gastric scintiscan, and manometry) usually in children with atypical manifestations of the disease.[5 8 12]

Our findings showed that 24 hour pH monitoring has high sensitivity and specificity, confirming the work of other authors[1 2]; we believe that it should be used as the first line of investigation in the diagnosis of gastro-oesophageal reflux.

Contrary to Schlesinger *et al*[13] we did not find any false negatives, so we conclude that a negative result must be thought of as being sufficient to rule out gastro-oesophageal reflux. This avoids further less sensitive and specific as well as potentially more harmful investigations such as barium swallow examination. In our experience the 24 hour pH monitoring was easy to do, well tolerated, and free from complications.

We believe that a barium swallow examination or oesophagoscopy, or both, are justified after a positive pH study or when an upper gastrointestinal malformation, an oesophageal stenosis, or oesophagitis are suspected. Gastric scintiscan should be used to detect bronchopulmonary aspiration.

After a diagnosis of gastro-oesophageal reflux has been reached, the therapeutic options (especially the indications for operation) must be considered.

Most authors agree that the clinical picture is of utmost importance in the therapeutic approach. The presence of serious symptoms or complications (such as severe oesophagitis with or without associated oesophageal stenosis, apnoeic spells, aspiration), or anatomical defects (such as severe hiatal hernia and previous oesophageal atresia) are often considered to be indications for operation.[3–8]

Apart from these, operation may become necessary after the failure of medical treatment, which according to different authors can be given for a period of time varying from six weeks[6] to three years.[14]

The age of the patients should also be considered. In the cases we studied the indication for operation was much higher among children older than 18 months of age (9/13, 69%) as opposed to those who were younger (6/23, 26%). This confirms previous work[15] and is in accordance with the natural spontaneous improvement of the disease up to 18 months of age.[8 16]

Surgery is particularly indicated in children with central nervous system abnormalities in which vomiting, poor growth, and pulmonary complications are important, and who usually respond poorly to medical treatment.[5 12 14] In our study there were only four patients with such abnormalities and two of them had operations.

The role of diagnostic procedures in identifying patients at particular risk for surgery is not well documented, but most of them seem to be useless. In a recent study the presence of severe oesophagitis confirmed histologically was reported as having a prognostic value in identifying those patients in whom medical treatment was most likely to fail.[17]

Few and contradictory data have been published about the use of prolonged oesophageal pH probing in predicting which patients will need operation. In 1986 Koch reported that 24 hour oesophageal pH probing is useless in the prediction of which patients need operation.[18] In the same year Ramenofsky *et al* found a significant correlation between a low number of episodes of reflux (less than 38–43) and the success of medical treatment.[19] Evans *et al* showed that a frequency duration index (calculated, before starting medical treatment, by multiplying the cumulative duration/hour by the mean frequency of reflux/hour) was significantly lower in the medically treated patients than in those who subsequently underwent operation.[20]

Our findings also suggest the usefulness of 24 hour pH monitoring in order to identify those patients who will not benefit from medical treatment. We studied the 'classical' measurements described by DeMeester *et al.*[1] Among them the more significant and those with the least overlap between medical and surgical patients were the percentage of reflux time and the number of episodes of reflux lasting more than five minutes in 24 hours. All patients with a percentage of reflux time higher than 27, or with more than 20 episodes of reflux lasting more than five minutes, or both, needed operation. In addition, the total number of episodes of reflux in 24 hours was significantly greater in the surgically treated patients than in those who received medical treatment even though, in contrast to the data presented by Ramenofsky *et al*,[19] we found considerable overlap between the two groups.

Our findings therefore show that patients with a percentage of reflux time greater than 27, or more than 20 episodes of reflux lasting more than 5 minutes, or both, have little chance of improving on medical treatment. In such patients medical treatment should, however be given for a short period, certainly less than a month.

To come to definitive conclusions about the role of oesophageal pH monitoring in predicting the need for operation, a larger number of patients should be studied. Nevertheless our data suggest that the pH probing should be used together with clinical data or other investigations, or both, to identify those children who will not respond to medical treatment, but rigid criteria cannot yet be set.

References

[1] DeMeester TR, Wang CI, Wernly JA, *et al.* Technique, indications and clinical use of 24 hour esophageal pH monitoring. *J Thorac Cardiovasc Surg* 1980;**79:**656–70.

[2] Schindlebeck NE, Heinrich C, Konig A, Dendorfer A, Pace F, Muller-Lissner A. Optimal thresholds, sensitivity and specificity of long term pH-metry for the detection of gastroesophageal disease. *Gastroenterology* 1987;**93:**85–90.

[3] Johnson DG, Herbst JJ, Oliveros MA, Steward DR. Evaluation of gastrooesophageal reflux surgery in children. *Pediatrics* 1977;**59:**62–8.

[4] Leape LL, Ramenofsky ML. Surgical treatment of gastroesophageal reflux in children. *Am J Dis Child* 1980;**134:**935–8.

[5] Spitz L, Kirtane J. Results and complications of surgery for gastro-oesophageal reflux. *Arch Dis Child* 1985;**60:**743–7.

[6] Johnson DG, Jolley SG, Herbst JJ, Cordell LL. Surgical selection of infants with gastroesophageal reflux. *J Pediatr Surg* 1981;**16:**587–94.

[7] Davidson BR, Hurd DM, Johnstone MS. Nissen fundoplication and pyloroplasty in the management of gastro-esophageal reflux in children. *Br J Surg* 1987;**74:**488–90.

[8] Sheperd RW, Wren J, Evans S, Lander M, Ong TH. Gastroesophageal reflux in children: clinical profile course and outcome with active therapy in 126 cases. *Clin Pediatr* 1987;**26:** 55–9.

[9] Strobel CT, Byrne WJ, Ament ME, Euler AR. Correlation of esophageal length in children with height: application to the Tuttle test without prior esophageal manometry. *J Pediatr* 1979;**94:**81–4.

[10] Arasu TS, Wyllie R, Fitzgerald JF, *et al.* Gastroesophageal reflux in infants and children: comparative accuracy of diagnostic methods. *J Pediatr* 1980;**96:**798–803.

[11] Cleveland RH, Kushner DC, Schwartz AN. Gastroesophageal reflux in children: results of a standardized fluoroscopic approach. *AJR* 1983;**141:**53–6.

[12] Byrne WJ, Euler AR, Ashcraft E, Nash DG, Seibert JJ, Golladay ES. Gastroesophageal reflux in the severely retarded who vomit: criteria for and results of surgical intervention in twenty-two patients. *Surgery* 1982;**91:**95–8.

[13] Schlesinger PK, Donahue PE, Schmid B, Layden TJ. Limitations of 24-hour intraesophageal pH monitoring in the hospital setting. *Gastroenterology* 1985;**89:**797–804.

[14] Schmitt M, Pfeiffer B, Pierre E, Barthelme H. L'intervention de Nissen chez l'enfant encephalopathe. *Chir Pediatr* 1986;**27:** 138–42.

[15] Boix-Ochoa J. Address of honored guest: the physiological approach to the management of gastric esophageal reflux. *J Pediatr Surg* 1986;**21:**1032–9.

[16] Carre J. The natural history of the partial thoracic stomach ('hiatal hernia') in children. *Arch Dis Child* 1959;**34:**344–8.

[17] Hyams JS, Ricci A, Leichter AM. Clinical and laboratory correlates of esophagitis in young children. *J Pediatr Gastroenterol Nutr* 1988;**7:**52–6.

[18] Koch AW. Extended pH monitoring in the evaluation of gastroesophageal reflux in infancy and childhood. *Pediatric Surgery International* 1986;**1:**161–7.

[19] Ramenofsky ML, Powell RW, Curreri PW. Gastroesophageal reflux: pH probe directed therapy. *Ann Surg* 1986;**206:**531–6.

[20] Evans DF, Haynes J, Jones JA, Stower MJ, Kapila L. Ambulatory esophageal pH monitoring in children as indicator for surgery. *J Pediatr Surg* 1986;**21:**221–3.

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Spinal deformity in patients born with oesophageal atresia and tracheo-oesophageal fistula

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SUMMARY Spinal deformity was present in 58 (19%) of 302 patients born with oesophageal atresia and fistula when examined at review. This was present in 24 (47%) of 51 patients with vertebral anomalies and 34 (14%) of 251 patients with normal vertebrae. Scoliosis was present in 21 patients with vertebral anomalies, torticollis in two, and lordosis in one. Two thirds of the patients with congenital scoliosis have had or are likely to require operation, compared with five patients with non-congenital scoliosis. Scoliosis associated with mixed vertebral anomalies in the lower thoracic spine had the worst prognosis. The medical records of a further 64 patients who had survived operation but who could not be traced were reviewed, and indicated that four had had congenital vertebral anomalies but none had a spinal deformity.

We recommend early detection of vertebral anomalies in this group and careful follow up of patients with these abnormalities.

Morbidity after repair of oesophageal atresia and fistula is not limited to respiratory[1] and surgical complications.[2] Spinal deformity has been reported in later childhood,[3–5] and may be related to associated congenital vertebral anomalies,[6–8] the thoracotomy,[3 4] and chronic pleural infection.[3] Previous reports of scoliosis in patients born with oesophageal atresia and tracheo-oesophageal fistula have been limited to small numbers and have excluded patients with congenital vertebral abnormalities.

The present report is a clinical and radiological review of 366 patients born with oesophageal atresia and tracheo-oesophageal fistula.

Patients and methods

Five hundred and thirty eight patients with oesophageal atresia and tracheo-oesophageal fistula have been treated at the Royal Children's Hospital, Melbourne, between 1948 when the first successful repair was carried out, and the end of 1985. Between August 1986 and April 1987, 302 of the 366 survivors were interviewed and examined as part of a long term follow up study. The patients were aged 1 to 37 years with a mean age of 18 years, and 59% were men or boys. Radiographs from the time of initial diagnosis as well as any performed subsequently were available for assessment in 333 patients, and radiological reports alone in the remaining 33. Each patient was examined for evidence of scoliosis, and their previous radiographs reviewed. Ten children and adolescents with scoliosis not previously recognised had new spinal radiographs. Twelve adults with minimal scoliosis did not have new radiographs as they had no symptoms referrable to the back. The Cobb angle was measured.[9] There were no data in the medical records of the 64 patients who were not examined to suggest that they had had scoliosis or other spinal deformity.

Vertebral anomalies were classified as: segmentation defects consisting of unilateral unsegmented bars and block vertebrae; formation defects consisting of complete (hemivertebrae) or partial defects (wedge vertebrae); or mixed defects consisting of unilateral failure of segmentation with contralateral hemivertebrae at the same level. Partial and complete sacral agenesis and absence of the coccyx were included in formation defects. Numerical rib anomalies were documented.

The scoliotic deformity was subdivided into an upper thoracic curve if the apex lay between the second and sixth thoracic vertebrae; a lower thoracic curve if it lay between the seventh and 11th thoracic vertebrae; a thoracolumbar curve if the apex was at the 12th thoracic or first lumbar vertebrae; a lumbar if it lay between the second and fourth lumbar vertebrae, and a lumbosacral if the apex was at the fifth lumbar vertebra.

Total lung capacity and vital capacity were measured in a pressure compensated plethysmograph (Jaeger Bodyscreen) in patients over the age of 6 who were able to cooperate. The best of a minimum of three efforts in the sitting position was recorded. Values were expressed as percent predicted for age, height and sex, based on the normal values of Zapletel *et al*[10] and Knudson *et al.*[11 12]

The unpaired Student's *t* test was used to analyse lung function and the χ^2 test to determine significance of differences between congenital vertebral abnormalities and other congenital abnormalities.

Results

Sixty seven congenital vertebral abnormalities were documented radiologically in 55 patients. They were equally distributed among the different types of oesophageal atresia but only one out of 26 patients with an isolated tracheo-oesophageal fistula had a vertebral anomaly. The types of anomalies present are given in table 1. Eleven patients had a combination of defects at different vertebral sites. Patients with anorectal and rectal anomalies were more likely to have vertebral anomalies ($p<0\cdot05$). Numerical rib abnormalities, present in 81 patients, were more common in those with vertebral abnormalities.

Spinal deformities were clinically apparent in 24 (47%) of the 51 patients with vertebral abnormalities who attended for review: scoliosis in 21 (confirmed radiologically), torticollis in two, and a lordosis in one. The types and distribution of anomalies in the 21 patients with scoliosis are given in table 2.

Table 1 *Types and distribution of congenital vertebral anomalies in 55 patients*

	Formation defects	*Segmentation defects*	*Mixed defects*
Cervical	0	3	0
Cervicothoracic	2	3	3
Upper thoracic	8	2	4
Lower thoracic	8	0	6
Thoracolumbar	1	3	2
Lumbar	1	2	1
Lumbosacral	6	3	0
Sacral	8	0	0
Coccygeal	1	0	0
Total	35	16	16

Table 2 *Sites of congenital scoliosis in patients with vertebral anomalies*

	Formation defects	*Segmentation defects*	*Mixed defects*
Total with scoliosis	10	4	7
Upper thoracic	1	2	1
Lower thoracic	5	0	5
Thoracolumbar	1	2	1
Lumbar	0	0	0
Lumbosacral	3	0	0

Surgical stabilisation of the spine was performed in eight patients: seven during adolescence and one in adult life. Mixed deformities in the lower thoracic spine were present in three of these, formation defects in the lower thoracic spine in two, a segmentation defect in the upper thoracic spine in one, a mixed defect in the thoracolumbar spine in one, and a formation defect in the thoracolumbar spine in one patient. Operation was not carried out in one adult patient with a segmentation defect and a 45° thoracolumbar curve because he was also mentally retarded. Five preadolescent children had significant curves (>20°); lumbosacral formation defects and curves of 20° and 27° were present in two, lower thoracic mixed defects and curves of 20° in two, and a segmentation defect and a curve of 26° in one patient.

A mixed defect and a segmentation defect were found in the cervicothoracic spine of the two patients with torticollis. The patient with the segmentation defect had a severe torticollis. A lumbosacral spondylolisthesis was present in one patient who presented with a lumbar lordosis and had had it operated on.

A thoracic scoliosis was present in 34 patients (14%) with normal vertebrae. An upper thoracic curve was present in 32 and a lower thoracic curve in two. Surgical stabilisation of the spine was done during adolescence in one patient with an upper thoracic curve. Curves of 20° and 25° were seen on the radiographs of two preadolescent children, and curves of between 15 and 20° in 17 adolescent and adult patients. A mild scoliosis was present on clinical examination of 12 other patients. Operation was carried out during adolescence in one girl with a lower thoracic curve and the other, a preadolescent girl, had a curve of 22°. Scoliosis at other sites was not found in patients with normal vertebrae.

Lung function is summarised in table 3. Patients with scoliosis had a reduction in total lung capacity and vital capacity compared with those with a normal spine ($p<0\cdot001$). Patients with congenital

Table 3 *Lung function results in 202 patients with repaired oesophageal atresia and tracheo-oesophageal fistula. Figures are expressed as mean % plus one standard deviation of predicted normal based on height*

	No scoliosis	*All scoliosis*	*Congenital scoliosis*	*Non-congenital scoliosis*
Total lung capacity	98·3+13·1	89·5+16·4	86·3+16·2	91·8+16·5
Vital capacity	89·5+16·4	75·8+19·2	74·3+21·4	76·8+17·7

scoliosis had a reduction of total lung capacity and vital capacity compared with patients with non-congenital scoliosis, but the difference was not significant.

Discussion

Vertebral anomalies and spinal deformity in patients born with oesophageal atresia and tracheo-oesophageal fistula are common. Congenital vertebral anomalies were present in 15% of patients in the present study, compared with an incidence of 2 to 45% in previous reports.[6 7 13 14] Those were based on small numbers or did not include specific radiological review. Our incidence is a minimal one as in 10% of patients we had only radiological reports and in some radiographs the whole spine was not shown. As previously reported, vertebral abnormalities were less common with isolated tracheo-oesophageal fistula, which is true of other anomalies as well.[1 15] The association of vertebral, renal, and anorectal anomalies is already well recognised and emphasises the importance of looking for latent renal abnormalities in patients with vertebral abnormalities.[16]

Vertebral anomalies cause an imbalance in the longitudinal growth of the spine caused by a deficiency either in the number of end plates, or in their rate of growth on one side of the spine.[17] The severity of the lateral curve that develops is proportional to the degree of the imbalance of growth. The formation defects were the most common type of anomaly causing scoliosis, and the lower thoracic spine the most common site. Two thirds of our patients with congenital scoliosis have had or are likely to require operation, compared with half quoted for congenital sciolosis.[18] Mixed defects in the lower thoracic spine seem to have the worst prognosis.[18 19]

Lumbosacral curves (as present in two of our patients) are potentially serious as there is no room below the vertebral defect for natural compensation to occur. This deformity is particularly difficult to correct.[20]

The prognosis for patients with scoliosis in the absence of congenital abnormalities is usually good. Operation was necessary, however, in two patients, and three children had pronounced curves that may progress. The aetiology probably has several components, but rib resection during thoracotomy,[21 22] scar formation from the incision itself,[23] and chronic pleural infection with rib fusion[5 24] may all contribute. Scoliosis is commoner in patients who have had several thoracotomies.[4] One patient requiring operation and one of the three with pronounced curves, however, had lower thoracic scoliosis of the type typical of idiopathic scoliosis. Given that the prevalence of scoliosis with curves greater than 20° at age 14 is 5/1000,[25] finding two in this group of 302 patients is not surprising.

We confirmed that patients with scoliosis had an appreciable reduction in lung volume. While anthropometric techniques have been standardised for normal children, this is not the case for patients with scoliosis. Clearly height becomes progressively more inappropriate as the severity of scoliosis increases and its use may underestimate the degree of abnormality. Many scoliosis clinics are now using the measurement of arm span to normalise lung function, but adequate normal data for these measurements in healthy children were not available at the time of the study.

Congenital and non-congenital spinal curves are common in patients born with oesophageal atresia and fistula. The thoracotomy may further contribute to the progression of the congenital scoliosis. Routine screening of the spine at birth for congenital anomalies and careful follow up if they are found are recommended. Some anomalies are associated with a poor prognosis and early treatment is recommended.[26] It is far better to prevent an increase in the deformity than to correct it once it has become severe.

References

1 Dudley NE, Phelan PD. Respiratory complications in long term survivors of oesophageal atresia. *Arch Dis Child* 1976;**51**: 279–82.

2 Myers NA. Oesophageal atresia and/or tracheo-oesophageal fistula. A study of mortality. In: Rickham PP, Hecker WC, Prevot S, eds. *Causes of postoperative death in children.* Baltimore: Urban and Schwarzenberg, 1979:141–65.

3 Durning RP, Scoles PV, Fox OD. Scoliosis after thoracotomy in tracheo-oesophageal fistula patients. *J Bone Joint Surg (Am)* 1980;**62**:1156–8.

[4] Chetcuti P, Myers NA, Phelan PD, Beasley SW, Dickens DRV. Chest wall deformity in patients with repaired oesophageal atresia. *J Pediatr Surg* 1989;**24**:244–7.

[5] Gilsanz U, Boechat IM, Birnberg FA, King JD. Scoliosis after thoracotomy for oesophageal atresia. *AJR* 1983;**141**:457–60.

[6] Louhimo I, Lindahl H. Oesophageal atresia primary results of 500 consecutively treated patients. *J Pediatr Surg* 1983;**18**:217–29.

[7] Holder TM, Cloud DT, Lewis JE, Pilling GP. Oesophageal atresia and tracheo-oesophageal fistula: a survey of its members by the surgical section of the American Academy of Pediatrics. *Pediatrics* 1964;**34**:542–9.

[8] Bond-Taylor W, Storer F, Atwell JD. Vertebral anomalies associated with esophageal atresia and tracheo-esophageal fistula with reference to the initial operative mortality. *J Pediatr Surg* 1973;**8**:9–13.

[9] Cobb JR. Outline for the study of scoliosis. In: Arbor A, Edwards JW, eds. *Instructional course lectures.* American Academy of Orthopaedic Surgeons, 1948;**5**:261–75.

[10] Zapletal A, Paul T, Samanell M. Die Bedeutung heutiger methoden der lungen funktion zur Feststelling einer obstruktion der atemarege bei Kinder und Jugendlichen. *Z Erkr Atmungsorgane* 1977;**149**:343–71.

[11] Knudson RJ, Slatin RL, Lebowitz MD. The maximal expiratory flow volume curve. Normal standards, variability and effects of age. *Am Rev Respir Dis* 1976;**113**:587–600.

[12] Knudson RJ, Lebowitz MD, Holberg CJ, Burrows B. Changes in the normal maximal expiratory flow-volume curves with growth and ageing. *Am Rev Respir Dis* 1982;**127**:125–34.

[13] Swenson O, Lipman R, Trisher JH, Deluca FG. Repair and complications of oesophageal atresia and tracheo-esophageal fistula. *N Engl J Med* 1962;**267**:960–3.

[14] Martin LW. Management of oesophageal anomalies. *Pediatrics* 1965;**36**:342–50.

[15] Schneider KM, Becher JM. The 'H-type' tracheo-oesophageal fistula in infants and children. *Surgery* 1962;**51**:677–86.

[16] MacEwan G, Winter R, Hardy J. Evaluation of kidney anomalies in congenital scoliosis. *J Bone Joint Surg (Am)* 1972;**54**:1451–4.

[17] Bick EM, Lopel JW. Longitudinal growth of the human vertebra. A contribution to human osteogeney. *J Bone Joint Surg (Am)* 1950;**32**:803–14.

[18] Winter RB, Moe JH, Gilers VE. Congenital scoliosis. A study of 234 patients treated and untreated. Part 1: Natural history. *J Bone Joint Surg (Am)* 1968;**50**:1–15.

[19] McMaster MJ, Ohtsuka K. The natural history of congenital scoliosis. A study of two hundred and fifty-one patients. *J Bone Joint Surg (Am)* 1982;**64**:1128–47.

[20] Winter RB. *Congenital deformities of the spine.* New York: Thieme-Stratton, 1983.

[21] Stauffer GS, Mankin HJ. Scoliosis after thoracoplasty. *J Bone Joint Surg (Am)* 1966;**48**:339–48.

[22] Loynes RD. Scoliosis after thoracoplasty. *J Bone Joint Surg (Am)* 1972;**54**:484–98.

[23] Shelton JE, Julian R, Walburgh E, Schneider E. Functional scoliosis as a long term complication of surgical ligation for patent ductus ateriosus in premature infants. *J Pediatr Surg* 1986;**21**:855–7.

[24] Bisgard JD. Thoracogenic scoliosis. Influence of thoracic disease and thoracic operations on the spine. *Arch Surg* 1934;**29**:417–45.

[25] Shands AR, Eisberg HB. The incidence of scoliosis in the State of Delaware. *J Bone Joint Surg (Am)* 1955;**37**:1243–9.

[26] Winter RB. Spinal problems in pediatric orthopaedics. In: Lovell WW, Winter RB, eds. *Pediatric orthopaedics.* Vol 2. Philadelphia: JP Lippincott, 1986:606.

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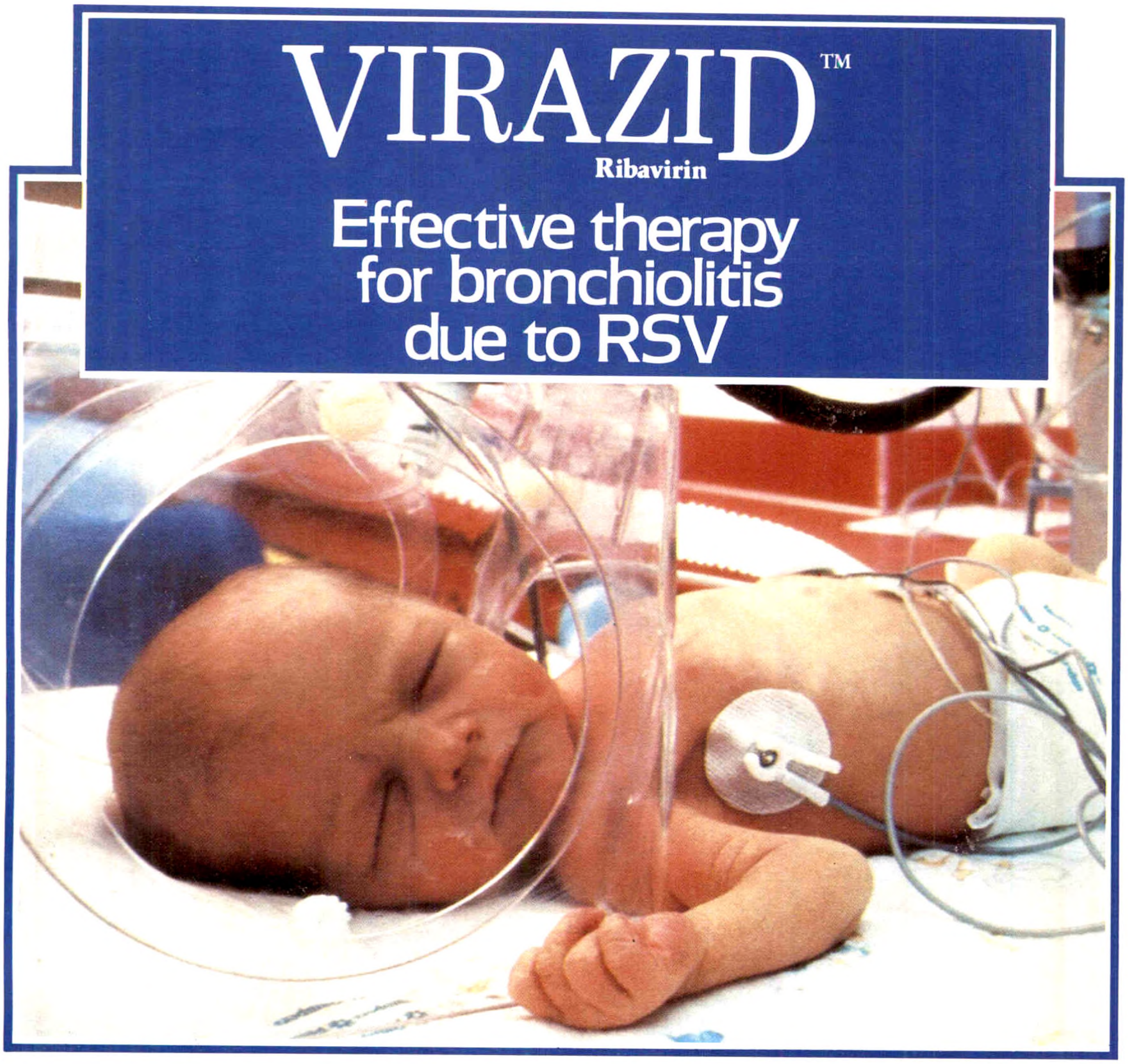

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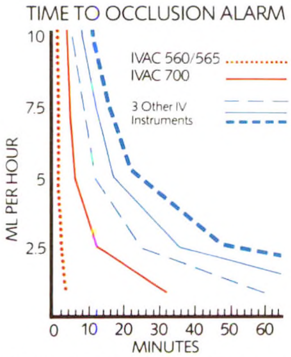

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Respiratory failure and mechanical ventilation in severe bronchiolitis

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SUMMARY A retrospective review of children who needed mechanical ventilation for severe bronchiolitis identified 62 cases over a 10 year period. The mean age at initiation of ventilation was 73 days (range: 14–201). Compared with a group of 150 children in hospital for bronchiolitis but not transferred to the intensive care unit, these 62 cases were significantly younger (73·0 compared with 166·3 days), and smaller (4·5 compared with 6·8 kg), and significantly more had been born prematurely (40% compared with 16%). Taken independently, age, weight, and prematurity were significantly associated with the need for artificial ventilation, weight being the most important factor. Using stepwise logistic regression, prematurity in itself added to the quality of the prediction but age did not. The mean duration of mechanical ventilation was 105 hours (range 2–381). Duration of ventilation was significantly longer in children with a low gestational age at birth and a positive familial history of atopy. There were no deaths, and no patient developed pneumothorax or pneumomediastinum. Mechanical ventilation is well tolerated and safe in acute bronchiolitis.

Viral bronchiolitis is one of the most common paediatric respiratory diseases and an important cause of admission to paediatric wards. Every child has a 1% to 2% risk of being admitted to hospital with bronchiolitis in the first year of life[1]; 5% of those will have acute respiratory failure[2] and about 1% will die.[1 3] It is 20 years since the first patients were treated with artificial ventilation,[2 4 5] but despite its widespread use, particularly for severe cases, there have been only a few recent reports on the subject and they have focused mainly on the technical aspects of mechanical ventilation.

The purposes of this review were to trace the evolution of the illness in children needing mechanical ventilation for a first episode of viral bronchiolitis and to find out their demographic characteristics. In the second part of the study, we compared the demographic data of the ventilated group with those of a control group consisting of patients admitted to hospital but not to the intensive care unit to evaluate the importance of factors such as age, prematurity, and weight as prognostic factors for the need for ventilation.

Patients and methods

The medical records of all patients who were admitted for treatment of bronchiolitis to the 24 bed paediatric intensive care unit of the Sainte-Justine Hospital in Montreal during the 10 year period 1 January 1976 to 31 December 1985 were retrospectively reviewed. Chart numbers were identified from the records of discharge diagnoses kept in the paediatric intensive care unit. Children with underlying cardiopulmonary abnormalities, serious congenital malformations, and immunodeficiency diseases were excluded, as it is difficult to assess retrospectively the relative effect of these factors on the course of severe bronchiolitis. Children who had had previous episodes of bronchiolitis were also excluded, as well as those not meeting the diagnostic criteria used in the present study.

CRITERIA FOR ENTRY INTO THE STUDY

The diagnostic criteria of bronchiolitis included tachypnoea, cough, chest retractions, prolonged expiratory time, pulmonary rales, and hyperinflation of the lungs on chest radiographs. Criteria for admission to the paediatric intensive care unit included at least one of the following signs: exhaustion caused by expiratory obstruction, greatly diminished air entry, cyanosis of lips and mucous membranes when breathing room air, respiratory pauses or apnoea, or both, and respiratory or metabolic acidosis. The diagnosis of respiratory insufficiency was based on both clinical and biochemical data including alteration of mental state, cyanosis when fractional inspiratory oxygen (FiO_2) was 0·4, frequent or prolonged episodes of apnoea, a rapid increase in carbon dioxide tension ($PaCO_2$), $PaCO_2$ greater than 10 kPa, or an oxygen tension (PaO_2) less than 7 kPa when FiO_2 was 1·0. A nosocomial infection was considered likely if the child was in hospital for a problem not associated with the respiratory system and developed wheezing seven days or more after the admission. Nosocomial infection was also considered when a child had been discharged and was readmitted with wheezing that developed less than three days after the initial discharge from the hospital. We defined prematurity as birth before 38 weeks' gestation, and a family history of atopy if the parents or siblings of the patient, or both, had a history of asthma, eczema, urticaria, or allergic rhinitis. It was assumed that if a finding (such as history of atopy) was not recorded in the chart, it was not present.

MANAGEMENT

All patients with acute respiratory insufficiency were ventilated with intermittent mandatory positive pressure ventilation by a volumetric (Bourns) or a time cycled (Sechrist) ventilator. Initial ventilatory settings were as follows: a peak inspiratory pressure of 25 to 35 cm H_2O — enough to achieve adequate chest expansion; a ventilatory rate of 25 to 30/minute; an inspiratory: expiratory ratio of 1:2 or longer; and a fraction of inspired oxygen of 0·5 to 0·7. Ventilatory settings were adjusted to keep the pH higher than 7·35, PaO_2 greater than 10 kPa and $PaCO_2$ less than 7 kPa. If hypercarbia worsened, and retractions and respiratory rate increased, delivered tidal volume was increased by raising the ventilatory rate to 35/minute, or the peak inspiratory pressure by increments of 3 to 5 cm H_2O to a maximum of 40–45 cm H_2O. As the patient was improving the ventilation rate was progressively decreased until the patient was breathing spontaneously with a continuous positive airway pressure of 2 to 3 cm H_2O. If the patient's condition remained stable for 4–12 hours on continuous positive airway pressure the patient was extubated and given oxygen with an oxygen hood. The duration of ventilation was defined as the total number of hours that the patient received positive pressure ventilation. All patients received intravenous hydration at maintenance rate (80–90 ml/kg/day) for some days. Sedative drugs (diazepam, morphine, or pethidine), muscular paralysis, theophylline derivatives, and antibiotics were used only when indicated. Patients given one dose or more of these agents were considered to have received the drug. No patients were given diuretics or ribavirin.

LABORATORY INVESTIGATIONS

The initial investigations on admission to the intensive care unit included a complete blood count and a chest radiograph. Arterial blood gases were measured regularly before intubation, during mechanical ventilation, and as long as the pulmonary condition was thought to be unstable. Microbiological cultures were taken from the endotracheal site at the time of intubation, regularly twice a week as long as the patient remained intubated and more often if infection was suspected. Blood was cultured if bacteraemia was suspected. Viral cultures of throat, stools, and tracheal secretions, and viral serology (paired samples for serum for neutralising antibodies for respiratory syncytial virus, influenza, parainfluenza, and adenovirus) were routinely done only during the last three years of the period reviewed. Washes of endotracheal secretions were inoculated onto HEp2 cell lines especially sensitive for respiratory syncytial virus and onto cynomolgus monkey kidney, and human foreskin fibroblast cell lines, for the detection of other viruses. Pulmonary superinfection was diagnosed if persistent fever and leucocytosis (white cell count $>15·0\times10^9/l$) were associated with purulent tracheal secretions and signs of pulmonary infiltration on chest radiographs. No pulmonary function tests were done.

CONTROL PATIENTS

To characterise patients requiring assisted ventilation more clearly, we analysed data from a control group of children with bronchiolitis who were admitted to hospital but not transferred to the intensive care unit. This group comprised infants and children who did not have underlying cardiopulmonary abnormalities, serious congenital malformations, or immunodeficiency diseases, and who were in hospital for the first time with bronchiolitis. Fifteen patients were chosen randomly (one of every five patients admitted was selected) by the medical records department for each of the 10 years of the study among all children admitted to our institution with this diagnosis; they were not matched with the ventilated group for age, sex, race, or month of admission.

STATISTICAL ANALYSIS

To compare clinical characteristics between ventilated and non-ventilated children, data were analysed by Student's *t* test, χ^2 with Yates's correction, and Fisher's exact test as appropriate. To determine the associations between duration of mechanical ventilation and age at admission, gestational age at birth, and PaCO$_2$ before intubation, we calculated Pearson correlation coefficients by simple linear regression analysis. To determine the associations between duration of ventilation and familial history of atopy, infiltration on initial chest radiograph, new lung infiltrations during ventilation, and use of sedatives, the two tailed Mann-Whitney U test was used because the data were not normally distributed. Only p values of 0·05 or less were regarded as significant. The Bonferroni correction was used to account for multiple testing at a p value of ≤0·005. Stepwise logistic regression analysis was used to examine the relative contribution of the three following variables to the need of artificial ventilation: weight, age, and prematurity.[9] The figures are given as mean (SD).

Results

Eighty one patients required assisted ventilation for severe bronchiolitis during the 10 year period reviewed. Nineteen children were excluded because of underlying pulmonary disease (n=8), congenital heart disease (n=3), multiple congenital malformations (n=2), and previous episodes of bronchiolitis (n=6). Sixty two patients were therefore included in the review. The male:female ratio was 1·3:1 (table 1) and the racial distribution was 53 white, eight black, and one Eskimo. The mean age at initiation of assisted ventilation was 73 days (range 14–201). Twenty five (40%) were born prematurely. Only four had had neonatal respiratory distress syndrome and three of these required mechanical ventilation at birth; two had had transient tachypnoea of the newborn. Twelve (19%) were thought to have acquired a nosocomial respiratory infection.

Among the comparative group of 150 children treated on general wards, the male female ratio was 2·1:1. The racial distribution consisted of 137 white, 12 black, and one Eskimo. These patients were significantly larger and older compared with the ventilated group (table 1) and the proportion of very young children (less than 2 months of age) significantly smaller (19% compared with 48%, p<0·0001). Prematurity was also encountered more often in the ventilated group, although the prevalence of respiratory distress syndrome and mechanical ventilation during the neonatal period was the same for both groups (table 1).

Stepwise logistic regression analysis was used to determine the relative effects of weight, age, and prematurity on the need for mechanical ventilation in the course of bronchiolitis. Taken independently each of these three factors was significantly related to the need for ventilation (p<0·001); weight, however, was the most significant factor determining the need for artificial ventilation. When weight was entered as the first step of the analysis (table 2), prematurity was associated with the need for mechanical ventilation, but age was not. When weight and prematurity were analysed together (second step of the analysis), age was not retained as a significant variable.

In the ventilated group the mean duration of symptoms of upper respiratory tract infection was 1·5 days before the onset of dyspnoea, and four days

Table 1 *Clinical data of ventilated and non-ventilated children with bronchiolitis*

	Ventilated group (n=62)	*Control group (n=150)*	*Statistical test*	*p Value*	*Relative risk (95% confidence interval)*
Age on admission (days):					
Mean (SD)	73·0 (41·4)	166·3 (139·7)	Mann-Whitney U	<0·001	
Median (range)	62 (14–215)	133 (16–988)			
Weight on admission (kg):					
Mean (SD)	4·5 (1·2)	6·8 (2·2)	Mann-Whitney U	<0·001	
Median (range)	4·2 (2·5–7·8)	6·6 (2·8–16·7)			
Gestation (weeks):					
No (%) <32	9 (14)	4 (3)	χ^2	<0·001	6·2 (1·9 to 20·1)
No (%) 32–37	16 (26)	20 (13)	χ^2	0·046	2·3 (1·0 to 5·1)
No (%) ≥38	37 (60)	126 (84)	χ^2	<0·001	0·3 (0·4 to 0·6)
Sex:					
Male:female	35:27	102:48	χ^2	0·11	
No (%) with neonatal respiratory distress syndrome	4 (7)	5 (3)	Fisher's exact	0·21	
No (%) who had mechanical ventilation during neonatal period	3 (5)	3 (2)	Fisher's exact	0·15	
No (%) with family history of atopy	12 (19)	49 (33)	χ^2	>0·05	

Table 2 *Stepwise logistic regression analysis to examine the relative contribution of weight, age, and prematurity to the need for mechanical ventilation*

	Coefficient	*Standard error*	*Coefficient/standard error*	*F value* — *To enter/to remove*	*df*	*p Value*
Step 1:						
Weight	−0·801	0·141	−5·664	— /33·99	1,189	0·017
Prematurity	—	—	—	5·75/ —	1,189	<0·0001
Age	—	—	—	0·14/ —	1,189	0·7128
Constant	3·4673	0·717	4·834	— /24·76	1,189	<0·0001
Step 2:						
Weight	−0·761	0·143	−5·317	— /29·03	1,188	<0·0001
Prematurity	0·458	0·201	2·274	— /5·31	1,188	0·022
Age	—	—	—	1·26/ —	1,188	0·263
Constant	3·4763	0·732	4·747	— /23·14	1,188	<0·0001

before mechanical ventilation was needed. Among initial laboratory studies, the mean haemoglobin concentration was 113 (2·5) g/l (range 74–180). The mean peripheral leucocyte count was 10·8 (2·6)×10^9/l (range 4·9–21·0). Arterial blood gases were analysed in 56 (90%) patients just before intubation; the mean pH was 7·20 (0·10) range 6·90–7·36, PaCO$_2$ was 9·6 (2·0) kPa, range 6·4–16·0, and mean bicarbonate was 27·4 (4·4) mmol/l, range 14·2–35·4. It should be noted that many children deteriorated suddenly and unexpectedly, which could explain why some infants had acceptable acid-base values before ventilation as measurements were made when the clinical condition seemed to be stable. In six cases (10%) the condition worsened so fast that the child had to be intubated immediately without time to sample blood gases. The initial chest radiographs showed at least one infiltrate in 33 of 62 patients (61%), and 21 (34%) had hyperinflation alone with or without increased bronchial markings.

Fifty patients were ventilated with Bourns ventilators and 12 with Sechrists. Duration of ventilation was 105 (79) hours, range 2–381, median 86. Seventeen patients (27%) were ventilated for 48 hours or less, and 19 (31%) between 49 and 96 hours.

There were no significant correlations between duration of mechanical ventilaton and age at admission (r=0·0; p=0·782), PaCO$_2$ before intubation (r=0·1; p=0·615), or the presence of infiltration on the initial chest radiograph (table 3). Low gestational age at birth correlated significantly with duration of ventilation (r=0·3; p=0·017). Three other factors also correlated with the length of ventilation: positive family history of atopy, appearance of new infiltrates during ventilation, and the use of sedative (table 3).

Most children tolerated the mechanical ventilation well; sedation was used in only 17 of 62 (27%) and muscular paralysis in two of 62 (3%). Theophylline or its derivatives were given in six of 62 (10%) of cases. Antibiotics were given to 56 of 62 of the patients (90%), mainly because of an infiltrate on the chest radiograph. Microbiological cultures from blood, cerebrospinal fluid, and urine done during the period of ventilation were sterile. Tracheal secretions yielded one or more potential pathogens in 14 patients. We believe that in several cases these positive tracheal cultures represented colonisation rather than true infection; there was evidence of pulmonary superinfection in seven of 62 (11%).

Viral cultures and serology were done in 43 and 25 ventilated patients, respectively. Respiratory syn-

Table 3 *Potential prognostic factors associated with duration of mechanical ventilation in 62 infants*

	Mean (SD) duration of mechanical ventilation (hours)	*p* Value*
Family history of atopy:		
Yes (n=12)	154·7 (85·3)	0·006
No (n=50)	93·6 (73·4)	
Infiltration seen on first chest radiograph:		
Yes (n=38)	93·3 (62·1)	>0·05
No (n=24)	124·5 (98·6)	
Occurrence of new lung infiltration during ventilation:		
Yes (n=22)	139·2 (75·8)	0·002
No (n=40)	86·8 (75·2)	
Sedation:		
Used (n=17)	127·4 (60·3)	0·02
Not used (n=45)	97·1 (84·1)	

*Two failed Mann-Whitney U test.

cytial virus was isolated by culture of tracheal or nasopharyngeal secretions in 12 patients, influenza A and adenovirus in one each. A fourfold or greater rise in antibody titres to respiratory syncytial virus occurred in 10 patients, and for parainfluenza 3 titres in one.

Complications were uncommon. There were no deaths and no patient developed pneumothorax or pneumomediastinum. New infiltrations were seen on chest radiographs in a third of intubated patients. In most cases they were not accompanied by fever, leucocytosis, or purulent tracheal secretions, and were considered to be of mechanical rather than infectious origin. Length of stay in the hospital after assisted ventilation had been stopped was 12·4 (12·4) days, range 3–80.

Discussion

Young age is often mentioned as a marker of severity in acute bronchiolitis.[10 11] The higher mortality in younger patients[11] can at least in part be associated with a higher rate of congenital malformations or of associated cardiopulmonary problems in this age group. Patients with these underlying conditions were not included in this review, so our results objectively confirm previous data showing that younger age is in itself a significant prognostic factor for acute respiratory failure in bronchiolitis.[11] Heycock and Noble attributed this phenomenon to the comparatively greater degree of obstruction produced by exudate or mucosal oedema in small calibre bronchi[11]; this is surely the principal explanation for the association between young age and severity of bronchiolitis. Our data offer objective support for this hypothesis: using a stepwise logistic regression analysis, we found that weight was the most significant factor determining the need to be mechanically ventilated. We believe that young infants are at higher risk of developing respiratory insufficiency during an episode of acute bronchiolitis, mostly because they are smaller and thus have smaller airways. Only a prospective study, however, can assess the importance of each risk factor.

Prematurity has recently been mentioned as one of the conditions that seems to place a child at risk of severe or fatal respiratory syncytial virus infections.[12] To date, however, only the likelihood of developing apnoea in the course of such an infection has been reported in infants who were born prematurely.[13 14] Our data show clearly that prematurity also predisposes to respiratory insufficiency secondary to acute bronchiolitis. Five of the seven children described by Joly *et al* (71%),[4] six of 15 of the patients reported by Outwater and Crone (40%),[6] and 14 of 36 described by Floret and Gabai (39%),[8] were also born prematurely; most of them had otherwise normal neonatal histories.

It is tempting to see a simple relation between the increased incidence of prematurity and the significantly lower mean weights in the ventilated compared with non-ventilated patients (table 1). Being smaller, infants who are born prematurely could be particularly at risk of developing severe bronchiolitis because of the smaller lumen of their bronchi, this explanation is possibly the most likely. None the less, the stepwise logistic regression analysis used to study the relative effects of weight, age, and prematurity showed that prematurity in itself adds to the quality of the prediction. Moreover, low gestational age was significantly correlated to the duration of mechanical ventilation.

Prematurely born children who do not have the respiratory distress syndrome have lower expiratory flow rates six to 12 years after birth than do children born at full term.[15 16] Bertrand *et al* found an increased incidence of airway hyper-reactivity in babies born prematurely and their families.[16] They suggested a possible association between the onset of premature birth and airway hyper-reactivity. This increased incidence of airway hyper-reactivity in prematurely born babies could predispose them to more severe bronchiolitis and acute respiratory failure. The duration of artificial ventilation was longer in those in our group of patients who had a family history of atopy, but the percentage of children with a family history of atopy was not significantly higher in the ventilated than in the non-ventilated groups (table 1). At present we do not know enough to explain these observations satisfactorily. Immunological considerations must also be kept in mind. Infants born at full term are immunodeficient when compared with older children and adults in essentially all measurable immunological factors; preterm infants are further immunocompromised, making them more susceptible to life threatening infections,[17] and possibly also to lower respiratory infections, as suggested by Ballow *et al.*[18]

Our review, as well as the reports of others,[6–8] show that mechanical ventilation for children with bronchiolitis is a well tolerated procedure. Only 27% of our patients required sedation. We believe that the decision to use sedatives should be based on the patient's course, and that muscular paralysis should only be used to ensure adequate gas exchange.

Serious complications such as death, pneumothorax, or pneumomediastinum are unusual in patients who do not have underlying cardiopulmonary diseases or immunodeficiency. Such complications did not occur in our series. Frankel *et al* described one case of pneumothorax and one case of acute pulmonary hypertension in 17 ventilated patients.[7] In another series reporting 36 ventilated infants, two developed pneumothoraces and another had an unexplained cardiac arrest.[8] Management of bronchiolitis in our intensive care unit has not changed appreciably over the period reviewed. Except for the use of ribavirin, principles of treatment have remained the same over the last 15 years.[19]

Except for low gestational age at birth and a family history of atopy, we were not able to identify other predictive factors for a long period of mechanical ventilation. There was an association between prolonged ventilation and the appearance of new lung infiltrates on chest radiographs. If most of these infiltrates had been associated with other clinical signs of infection we could speculate that bacterial pulmonary superinfection prolongs mechanical ventilation. This was not the case, however. Most of the new pulmonary densities were discovered on routine radiographs, and most were considered to be atelectasis. These infiltrates were probably a consequence of prolonged ventilation rather than a cause. Duration of ventilation was also longer in patients who received sedatives. These children were possibly the illest and the most difficult to ventilate, although this hypothesis is impossible to confirm retrospectively. We may also speculate that the use of sedation prolonged weaning from the ventilator, particularly in young children who are especially sensitive to the depressive effects of narcotics.

Bacterial infections do not seem to have a serious role in the course of acute bronchiolitis.[20] A prospective randomised study has not shown any benefit from the use of antibiotics in children admitted to hospital with bronchiolitis.[21] Nearly 90% of children in our ventilated group were given antibiotics. In most cases the antibiotics were prescribed for pulmonary infiltrates seen on the chest radiographs of critically ill, very young children. This attitude is understandable but one must realise that the bacterial infection rate is low.

Despite current recommendations,[12] enthusiastic acceptance of ribavirin for treatment of respiratory syncytial virus infections must still be questioned.[22 23] The efficacy of such treatment, especially in intubated patients, remains unproved.[23] We have shown that mechanical ventilation is an efficacious and well tolerated procedure for children who do not have underlying cardiac or pulmonary disease. This must be taken into consideration when defining for whom specific treatment might be cost effective.

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References

[1] Wohl MEB, Chernick V. Bronchiolitis. *Am Rev Respir Dis* 1978;**118**:759–81.

[2] Phelan PD, Williams HE, Stocks JG, *et al.* Artificial ventilation in the management of respiratory insufficiency in acute bronchiolitis. *Aust Paediatr J* 1968;**4**:223–8.

[3] Hicks RA, Taylor JW, Jackson MA, Burry F, Olson LC. Epidemiology of a large respiratory syncytial virus (RSV) outbreak in Kansas City, Missouri. *Pediatr Res* 1986;**20**:228A.

[4] Joly JB, Huault G, Amsili J, Lemerle J, Thieffry S. Place de l'intubation et de la ventilation artificielle dans le traitement des bronchoalvéolites graves du nourrisson. *Arch Fr Pediatr* 1967; **24**:303–18.

[5] Downes JJ, Wood DW, Striker TW, Haddad C. Acute respiratory failure in infants with bronchiolitis. *Anesthesiology* 1968;**29**: 426–34.

[6] Outwater KM, Crone RK. Management of respiratory failure in infants with acute viral bronchiolitis. *Am J Dis Child* 1984;**138**: 1071–5.

[7] Frankel LR, Lewiston NJ, Smith DW, Stevenson DK. Clinical observations on mechanical ventilation for respiratory failure in bronchiolitis. *Pediatr Pulmonol* 1986;**2**:307–11.

[8] Floret D, Gabai E. La ventilation assistée des bronchiolites aiguës du nourrisson. *Pediatrie* 1986;**41**:189–95.

[9] Dobson AJ. *An introduction to statistical modeling.* New York: Chapman and Hall, 1983:74–90.

[10] Glezen WP, Pareder A, Allison JE, Taber LH, Frank AL. Risk of respiratory syncytial virus infection for infants from low-income families in relationship to age, sex, ethnic group, and maternal antibody level. *J Pediatr* 1981;**98**:708–15.

[11] Heycock JB, Noble TC. 1,230 cases of acute bronchiolitis in infancy. *Br Med J* 1962;**ii**:879–81.

[12] Committee on Infectious Diseases: American Academy of Pediatrics. Ribavirin therapy of respiratory syncytial virus. *Pediatrics* 1987;**79**:475–8.

[13] Bruhn FW, Mokrohisky ST, McIntosh K. Apnea associated with respiratory syncytial virus infection in young infants. *J Pediatr* 1977;**90**:382–6.

[14] Church NR, Anas NG, Hall CB, Brooks JG. Respiratory syncytial virus related apnea in infants. *Am J Dis Child* 1984; **138**:247–50.

[15] Coates AL, Bergsteinsson H, Desmond K, Outerbridge EW, Beaudry PH. Long-term pulmonary sequelae of premature birth with and without idiopathic respiratory distress syndrome. *J Pediatr* 1977;**90**:611–6.

[16] Bertrand JM, Riley SP, Popkin J, Coates AL. The long-term pulmonary sequelae of prematurity: the role of familial airway hyperreactivity and the respiratory distress syndrome. *N Engl J Med* 1985;**312**:742–5.

[17] Bernbaum J, Anolik R, Polin RA, *et al.* Development of the premature infant's host defense system and its relationship to routine immunizations. *Clin Perinatol* 1984;**11**:73–84.

[18] Ballow M, Cates KL, Rowe JC, Goetz C, Desbonnet C. Development of the immune system in very low birth weight (less than 1500 g) premature infants: concentrations of plasma immunoglobulins and patterns of infection. *Pediatr Res* 1986;**20**: 899–904.

[19] Chantarojanabiri T, Nichols DG, Rogers MC. Lower airway disease: bronchiolitis and asthma. In: Rogers MC, ed. *Textbook of pediatric intensive care.* Baltimore: Williams and Wilkins, 1987:199–235.

[20] Hall CB, Powell KR, Schnabel KC, Gala CL, Pincus PH. Risk of secondary bacterial infection in infants hospitalized with respiratory syncital viral infection. *J Pediatr* 1988;**113**:266–71.

[21] Friis B, Andersen P, Brenoe E, *et al.* Antibiotic treatment of pneumonia and bronchiolitis. A prospective randomised study. *Arch Dis Child* 1984;**59**:1038–45.

[22] Wald ER, Dashefsky B, Green M. In re ribavirin: a case of premature adjudication? *J Pediatr* 1988;**112**:154–9.

[23] Roy CG. Ribavirin. Ambivalence about an antiviral agent. *Am J Dis Child* 1988;**142**:488–90.

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Routine immunisation of preterm infants

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SUMMARY Fifty one preterm infants (26–36 weeks' gestation) were enrolled in a study of their immunological responses to diphtheria, tetanus, pertussis, and polio antigens eight to 12 weeks after their primary courses had been completed. Samples from 21 infants born at full term were also analysed. Many infants were able to start immunisation at 3 months of age. Premature infants who are immunised as soon as possible after 3 months of age develop adequate antibody responses.

Immunisation is one of the most effective means we have of protecting children against infection Preterm infants are a vulnerable group and very preterm infants with long term respiratory problems are particularly at risk from respiratory infections such as pertussis; it is therefore important to protect them from pertussis as early as possible. In the United Kingdom the Department of Health recommends that immunisation programmes start at 3 months of age, and the current guidelines also advise starting immunisation of preterm infants at that age.[1] The practice varies across the country with some authorities starting at three months after the actual delivery date, some at three months from expected date of delivery, and some taking an arbitrary figure in between.[2] In the United States, it is recommended that preterm infants begin their immunisations at the appropriate chronological age regardless of prematurity, but they comment that there is no research to justify this.[3]

There are good theoretical grounds for supposing that preterm infants will be able to mount immune responses comparable with those born at full term. Antibody production increases with age, but this is related to duration of exposure to the antigen after birth rather than postconceptional age.[4] [5] Studies on cell mediated immunity suggest that preterm infants behave similarly to those born at full term.[6] The small amount of work that has been done on routine immunisation of preterm infants supports this view. Smolen *et al* looked at antibody responses to polio virus vaccine after two months and four months in 37 preterm infants; they had similar levels of antibody to those born at full term.[7] Bernbaum *et al* studied 25 infants from 28 to 34 weeks' gestation given triple vaccine (diphtheria, tetanus, and pertussis) at the ages of 2, 4, and 6 months.[8] After the first injection fewer preterm infants had satisfactory antibody concentrations, but by the second and third injections there were no significant differences when compared with those born at full term.

In Nottingham the local recommendation is for preterm infants to start the immunisation programme three months from birth, whatever the period of gestation, or as soon as possible after this. We have followed up a series of preterm babies to monitor what actually happens and to look at their immune responses to diphtheria, tetanus, pertussis, and polio vaccines.

Subjects and methods

Preterm infants were selected in the neonatal period while still in hospital. About 20 infants were chosen between 32 and 36 weeks' gestation, and a further 20 between 28 and 32 weeks' gestation. As many babies as possible were recruited who were born at under 28 weeks' gestation. Cord blood was sampled where possible. Parents were asked if they would like to take part in the study and those agreeing were given a written explanation. Consent was obtained from the local ethics committee. The general practitioner and health visitor were informed. The immunisations were carried out as a routine in the normal system, starting at three months where possible, the second dose six to eight weeks later, and the third dose four to six months later. At about eight to 12 weeks after the third dose, a blood sample was taken from the baby for analysis of diphtheria, tetanus, pertussis, and polio antibodies. The sample was separated and serum was stored frozen.

Blood samples taken from children for a variety of reasons in hospital between the ages of 12 months and 24 months that would normally have been discarded as excess were saved in the laboratory to act as controls. Immunisation records of these children were scrutinised to select samples taken at about the same interval after the three immunisations as the preterm infants. Samples were not used if the child was preterm or if there was an illness that might affect the immune response.

LABORATORY ASSAYS

Polio virus antibodies
This assay was performed by mixing 50 μl volumes containing approximately 100 times the median tissue culture infective dose of each of the three Sabin poliovirus types, with an equal volume of a dilution of a serum sample. The samples were diluted in microtitre plates in the range 1/2 to 1/256. The plates containing the serum virus mixtures were incubated at 35°C for two to three hours before adding 0·1 ml of a suspension of 5×10^3 HEp2 cells and reincubating for five days. The end point was expressed as the dilution of serum estimated to neutralise the challenge virus in 50% of the wells in the plates.

Diphtheria and tetanus antitoxin
An enzyme linked immunosorbent assay (ELISA), comparable with that described by Melville-Smith *et al* was used to estimate antitoxin concentrations in international units (IU)/ml.[9] Human reference serum samples of known potencies previously assayed by in vivo neutralisation tests were included in each assay.

Pertussis antibodies
These were estimated by an ELISA similar to that described above but using the supernatant from centrifuged sonicated cells of *Bordetella pertussis* strain W28 at a concentration of 8 ng protein/ml as antigen. The human reference was a serum pool from normal children vaccinated who had been vaccinated against diphtheria, tetanus, and pertussis, which had a nominal concentration of 100 pertussis antibody units/ml. Pertussis antibodies were detected using alkaline phosphatase conjugated antihuman polyvalent immunoglobulins (raised against α, γ, and μ chains).

Results

Fifty preterm infants completed the study (22 from 32–36 weeks' gestation, 19 from 28–31 weeks' gestation, and nine either 26 or 27 weeks' gestation). Twenty one control samples were analysed.

Many of the infants were able to start their immunisations by 12–13 weeks of age (fig 1). There was an inevitable delay with some of the very sick infants. The longest delays in starting immunisation were for social reasons—for instance, difficulty in getting the child to the clinic. One child was delayed while uncertainty about possible fits was being resolved. Five children had their first dose in the neonatal unit, and two of these had all their immunisations in hospital. One child of 33 weeks' gestation, who was immunised at the age of 16 weeks, was described as 'jittery' after the first triple dose, and had pertussis omitted from the second and third doses. No other reactions were reported other than mild fever, irritability, and redness at the injection site.

There were no neonatal contraindications to pertussis vaccine in any of the children despite some having had complicated neonatal courses. All the children started immunisation with the triple antigen. Two children were diagnosed as having cerebral palsy (one probably spastic quadriplegia, one mild hemiplegia). Both these children were born at 27 weeks' gestation and it was thought that the benefit from pertussis immunisation outweighed any possible risk.

Two children (of 27 and 31 weeks' gestation, respectively) had had admissions for asthma in the first year of life. One child (of 26 weeks' gestation) had persistent failure to thrive and feeding difficulties. One child (of 29 weeks' gestation) had necrotising enterocolitis necessitating resection of bowel. One child (of 26 weeks' gestation) had a tracheo-oesophageal fistula and a hand anomaly.

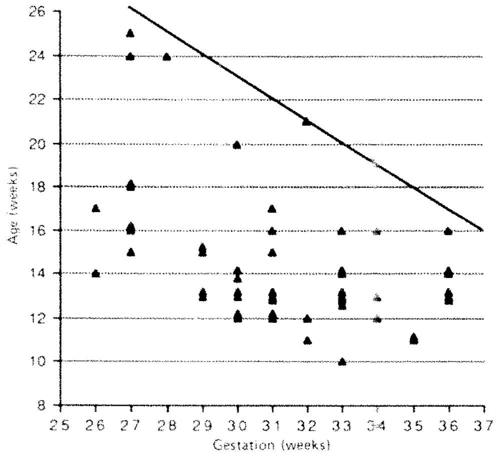

Fig 1 *Age of preterm infants at immunisation. The diagonal line indicates estimated date of delivery plus three months.*

Table *Immunological responses*

	Gestational age (weeks)			
	26–27 (n=9)	*28–31 (n=19)*	*32–36* (n=22)*	*≥37 (n=19)*
Diphtheria antitoxin (IU/ml):				
Mean (SD)	4·64 (3·2)	4·26 (3·6)	4·15 (3·1)	5·47 (6·03)
Range	0·42—10·73	0·42—12·97	0·55—12·12	0·49—25·92
Tetanus antitoxin (IU/ml):				
Mean (SD)	2·53 (1·2)	4·67 (3·4)	4·47 (2·2)	5·29 (7·4)
Range	0·59—4·26	0·45—13·6	1·73—9·9	0·40—34·38
Pertussis antibody (units/ml):				
Mean (SD)	31 (25·5)	73 (56)	48 (30)	69 (62)
Range	2—78	16—226	3—102	6—133

*One infant had diphtheria and tetanus only.

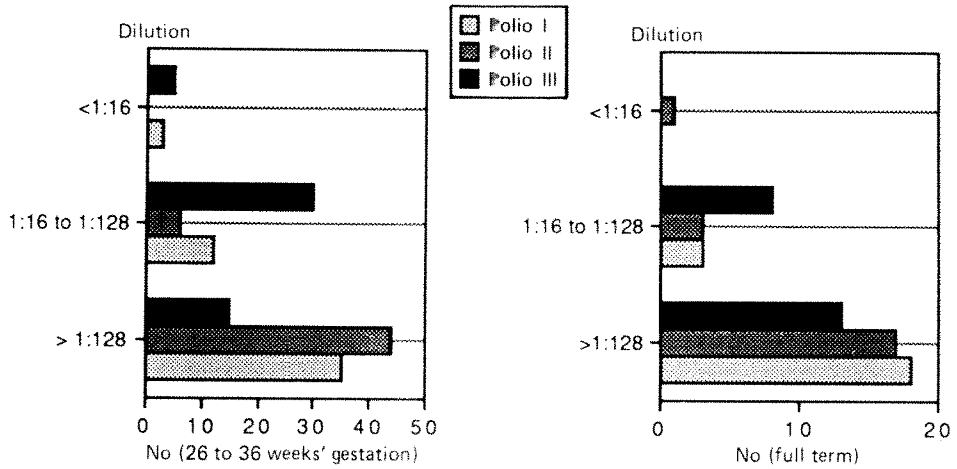

Fig 2 *Antibody production against polio immunisation, showing that premature infants responded as well as those born at full term.*

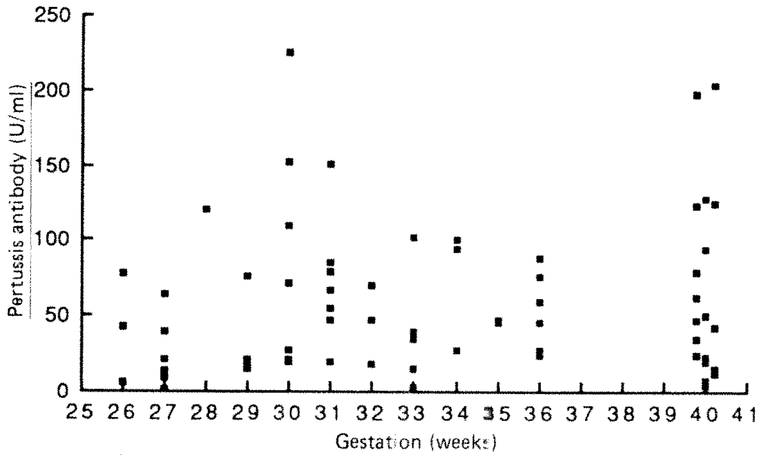

Fig 3 *Antibody production against pertussis vaccine in all infants. The circulating amount varied widely, but did not seem to be related to the infants' maturity at birth.*

Forty two of the children had blood samples taken for antibody measurements 8–12 weeks after the third immunisation. One child had the sample taken at one week, seven between 13–17 weeks, and one was 21 weeks after diphtheria, tetanus, and polio and five weeks after the pertussis (the third pertussis injection was delayed because of a question about contraindications). The immunological responses of all children were satisfactory to all antigens compared with the controls (table, fig 2). There was no relation between response and gestational age (individual values for pertussis are shown in fig 3) or the age of starting immunisations.

Cord blood samples were available for 19 children. There was no association between antibody concentrations in cord blood and the infant's response to immunisation, or between cord blood and gestation (though only two cord blood samples were from infants of less than 31 weeks' gestation).

Discussion

Infants born early are at high risk of serious consequences from pertussis because of their immaturity, their limited respiratory reserve, and possible residual damage after lung and brain problems related to their immaturity or neonatal care. Yet it is this group in whom the pertussis vaccine may be omitted from the immunisation programmes because of uncertainty about the contraindications, or in whom the programme is delayed as a deliberate policy or because of the delicate nature of the infants or uncertainty about their well being.

This investigation has shown that premature infants, vaccinated as soon as possible after 3 months of age, develop adequate responses as judged by circulating antibodies (comparable with those found in infants born at full term) and they did not develop more severe local reactions or show adverse systemic reactions. Inevitably the numbers are small, but they nevertheless support the 1988 DHSS recommendation that preterm infants should be immunised as soon as possible after 3 months.

Individual health staff within the community only rarely have to advise about immature infants. This may lead to a tendency to 'play safe' and postpone immunisation or even omit the pertussis immunisation altogether. A clear recommendation from the neonatal unit on neonatal contraindications to pertussis *and the lack of them*, and advice on the age at which to start, should help to overcome this.

We thank F Sheffield from the National Institute for Biological Standards and Control for help in setting up the study, and M Melville-Smith and D Magrath (also from the National Institute for Biological Standards and Control) for analysis of the samples.

References

[1] Joint Committee on Vaccination and Immunisation. *Immunisation against infectious disease*. London: DHSS, 1988.

[2] Lingham S, Miller C, Pateman J, *et al.* Immunisation of preterm infants. *Br Med J* 1986;**292:**1183–5.

[3] Report of the Committee of Infectious Diseases. *The 1986 red book*. 20th ed. Elk Grove Village: American Academy of Pediatrics, 1986.

[4] Rothberg RM. Immunoglobulin and specific antibody synthesis during the first weeks of life of premature infants. *J Pediatr* 1969;**75:**391–9.

[5] Bernbaum J, Andik R, Polin RA, Douglas SD. Development of the premature infant's host defence system and its relationship to routine immunisation. *Clin Perinatol* 1984;**11:**73–84.

[6] Wara DW, Barnet DJ. Cell mediated immunity in the newborn: clinical aspects. *Pediatrics* 1979;**64**(suppl):822.

[7] Smolen P, Bland R, Heiligenstein E, Lawless MR, Dillard R, Abramson J. Antibody response to oral polio vaccine in preterm infants. *J Pediatr* 1983;**103:**917–9.

[8] Bernbaum JC, Dart A, Audik R, *et al.* Response of preterm infants to diphtheria—tetanus—pertussis immunisations. *J Pediatr* 1985;**107:**184–8.

[9] Melville-Smith ME, Seagroatt VA, Watkins JT. A comparison of enzyme-linked immunosorbent assay (ELISA) with the toxin neutralisation test in mice as a method for the estimation of tetanus antitoxin in human sera. *J Biol Stand* 1983;**11:**137–44.

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Morbidity in whooping cough and measles

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SUMMARY Parents of 99 children who were admitted to hospital with whooping cough or measles, and of 50 children with whooping cough or measles who were nursed at home, were interviewed to determine the extent of morbidity and its effects on the family. Children admitted with whooping cough or measles spent a mean of 12·6 and 5·8 days in hospital, respectively. Time to full recovery was 13·7 and 2·1 weeks, respectively. Over a third of the children who were admitted were emotionally upset during the admission and for several weeks afterwards. Parental anxiety and exhaustion were common. Routine family life was appreciably disturbed. Advice from health care professionals, based on misconceptions of valid contradictions to immunisation, was the main reason for refusing vaccination.

Routine immunisation against whooping cough and measles is recommended for all except a small minority of preschool children.[1] The vaccines are safe and effective.[2 3] Failure to maintain high rates of vaccination results in epidemics[2 4 5] with preventable deaths and serious complications in previously normal children.[5-8] Even uncomplicated illness can cause substantial morbidity for both patient and family. Public confidence in whooping cough vaccine, eroded by a media campaign, is not reliably supported by the medical profession.[9] It is probable that the public extrapolates from its misinformation on pertussis vaccine to form erroneous concepts of the dangers of, and contraindications to, measles vaccine.[10 11] In 1985 the percentage of eligible children in England vaccinated before 3 years of age was 85% for diphtheria, tetanus, and polio but only 65% for pertussis and 68% for measles. The World Health Organisation's target is primary immunisation in 90% of all European children less than 2 years of age by 1990.

The aim of this study was to identify the morbidity associated with whooping cough and measles, the emotional and financial hardships that they cause within the family, and the areas where communication between health care professionals and the community has broken down.

Patients and methods

All children with whooping cough or measles admitted to Seacroft Hospital (which offers a service covering several districts to children with infectious diseases) from February 1986 to June 1987 were identified and examined by one of us. Mothers were asked at discharge if they would agree to be interviewed within the next six months about both their experience of the illness and their views on the childhood immunisation programme. Parents of 99 children were interviewed at home with a semistructured questionnaire. This had previously been assessed by the Leeds Health Education Service, firstly with a sample of people who understood only straight forward English, and secondly with teachers of English as a second language. The questions were designed to provide information on the immunisation state of the child, parental knowledge about the illnesses and the vaccines, reasons for failure to take up the immunisation, and the financial, emotional, and social impact of these preventable diseases on the family. Mothers of children notified to the Leeds medical officer of environmental health as having whooping cough or measles, and who were nursed at home, were also interviewed. All interviewees were selected randomly to provide a mix of social class. The study was approved by the hospital ethics committee.

Results

Seventy two children admitted with whooping cough, 27 admitted with measles, and 50 notified as suffering whooping cough (n=25) or measles (n=25) but not referred to hospital, were entered into the study. Overall, eight children (one admitted) were from social class I families, 27 (12 admitted) from class II, 53 (33 admitted) from class III, 15 (13 admitted) from class IV and 46 (40 admitted) from class V. Both interviewers obtained similar results.

The consequences of measles or whooping cough for the family and child are summarised in tables 1 and 2. The primary reasons for refusal of vaccination by parents of children eligible for immunisation are summarised in table 3. Only six of 97 cases of whooping cough had received pertussis immunisations (6%), whereas 26 (50%) of 52 children with measles had received measles immunisation. This included 19 (76%) of the 25 cases that were notified but treated at home.

The mothers of 10 children with whooping cough (7%), and 52 children with measles (35%) did not consider the illnesses to be serious. Ninety four (63%) felt that they had not received sufficient information about childhood immunisation. Further information requested was consistently: What is the disease? How do the side effects of the vaccine

Table 1 *Consequences of measles and whooping cough for the child and family*

	Whooping cough (n=97)	*Measles (n=52)*
No (%) of parents feeling exhaustion and having disturbed nights for up to 3 months	69 (71)	9 (17)
No (%) of parents with severe anxiety	43 (44)	5 (10)
No (%) of parents who feared that their child would die	28 (29)	1 (2)
Mean hospital stay and range (days)	12·6 (3–35)	5·8 (3–16)
Mean length (weeks) of illness (range)	13·7 (1 week–6 months)	2·1 (3 days–6 weeks)

Table 2 *General consequences of admission to hospital for the child and family (n=99)*

No (%) of parents who described their child as:	
Upset in hospital, clinging	37 (37)
Crying or demanding after discharge	38 (38)
No (%) of parents sleeping in hospital	49 (49)
Mean (range) time spent travelling to and from hospital each day by mother or father (hours)	1·4 (5 minutes to 6 hours)
Median (range) cost/day (£) spent in travel to and from hospital	1·6 (0–20)
No (%) of families of admitted children with their own car	44 (44)
Median (range) amount (£) spent on extra toys and sweets (n=28)	6 (2–56)
Median (range) number of parental working days lost as a result of child's illness (n=38)	5 (1–70)

Table 3 *Most common reasons for not accepting vaccination*

	Pertussis No (%)	*Measeles No (%)*
Fear of side effects	35 (37)	0
Family history of:		
Fits	32 (34)	7 (18)
Mental retardation	3 (3)	1 (3)
Atopy	10 (11)	7 (17)
Fits in the child	3 (3)	0
Parental apathy or disbelief in the value of immunisation	4 (4)	9 (23)
Child always had 'colds'	6 (7)	9 (23)
Spina bifida	1 (1)	0
Previously had target illness	0	3 (8)
Concurrent antibiotic treatment	0	1 (3)
No recall card received	0	2 (5)
Total	94 (100)	39 (100)

compare with the complications of the disease? How effective is the vaccine? Sixty parents (40%) had been influenced by adverse publicity. One hundred and forty three (96%) agreed with the principle of childhood immunisation.

Discussion

The severity of measles and whooping cough, though well documented,[5-8] is not consistently appreciated by either health care professionals[12] or parents.[13] Our study illustrates the extended family morbidity resulting from these illnesses (especially for pertussis) with parental exhaustion and severe anxiety being common. Considerable time and money are spent in visiting, working days are lost, and with 40% of parents sleeping in hospital with their children, social disruption at home is inevitable. These effects may be felt within the family for weeks or months. Thirteen (37%) of 35 social classes I and II children were admitted, compared with 53 (87%) of 61 from social classes IV and V. These socially disadvantaged families are perhaps least able to cope with the emotional and financial stress of having a child in hospital, and a child who may then need a long period of convalescence at home.

Acceptance of immunisation reflects belief in the efficacy and safety of the vaccine and realisation of the seriousness of the disease.[9] Misconceptions about the severity of childhood illness will gain credence from casual misdiagnosis. Three children had been diagnosed as having measles when less than 1 year of age, and were not subsequently given measles vaccine. A high proportion (68%) of such infants are seronegative to measles antigen.[14] Seventy six per cent of the 25 children with measles who were nursed at home had received measles vaccine. Many parents described a short lived feverish illness with a rash, but without conjunctivitis or cough. Misdiagnosis of other, less severe, viral infections as measles will perpetuate the myth that measles is a trivial illness. Diagnosis of measles in immunised children will erode faith in the efficacy of the vaccine. We suggest that when measles is diagnosed in children under 1 year old or who have previously been immunised, the diagnosis should be confirmed by serology.

The results of this study illustrate the many reasons given for non-immunisation, despite most parents' belief in the general principle of childhood immunisation. All our information was received from interviews with parents only, and it must be considered possible that their memories of contact with health care personnel, and of the advice offered to them, may be clouded by their own attitudes to immunisation, and feelings of guilt after their child had contracted a serious illness. None the less, the results of this study suggest that it is the health care professionals who—through advice based on misconceptions of the valid contraindications to immunisation—fail to protect children against these preventable infectious diseases. Our results show that the same misconceptions are being perpetuated that were recorded in earlier studies.[9 10] Nor is the profession consistent in the information it delivers to parents[15-17]; there are clear disparities in general practitioners' and health visitors' convictions about the need for whooping cough and measles vaccine compared with those for diphtheria, polio, and tetanus.[12 18]

Local[19] and national[20] studies have shown that professional commitment, publicity campaigns, and involvement of parents can raise the vaccination rate to the targeted 90% for primary immunisation of all European children less than 2 years of age. We have shown that there is extended morbidity for children and families during and after measles or pertussis infection. The target immunisation rate must be met.

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References

[1] Department of Health and Social Security. *Immunisation against infectious disease*. London: HMSO, 1988.

[2] Begg NT. Childhood vaccine—preventable disease. *Public Health Laboratory Service Microbiology Digest* 1987;**4:**38–40.

[3] Block AB, Orenstein WA, Stetles HC, *et al*. Health impact of measles vaccination in the United States. *Pediatrics* 1985;**76:** 524–32.

[4] MacKenzie AG. Children not immunised for measles. *Br Med J* 1985;**290:**1902.

[5] Swansea Research Unit of the Royal College of General Practitioners. Effect of a low pertussis vaccine uptake on a large community. *Br Med J* 1981;**282:**23–6.

[6] Miller CL. Severity of notified measles. *Br Med J* 1978;**i:**1253.

[7] Miller CL. Death from measles in England and Wales 1970–83. *Br Med J* 1985;**290:**443–4.

[8] Nicoll A, Gardner A. Whooping cough and unrecognised postperinatal mortality. *Arch Dis Child* 1988;**63:**41–7.

[9] Stevens D, Baker R, Hands S. Failure to vaccinate against whooping cough. *Arch Dis Child* 1986;**61:**382–7.

[10] Nicoll A. Contra-indications to whooping cough immunisation—myths or realities. *Lancet* 1985;**i:**679–81.

[11] Adjaye N. Measles immunisation. Some factors affecting non-acceptance of vaccine. *Public Health* 1981;**95:**185–8.

[12] Wilkinson P, Tylden-Patterson L, Gould J. Professional attitudes towards vaccination and immunisation within the Leeds Area Health Authority. *Public Health* 1979;**93:**11–5.

[13] Blair S, Shave N, McKay J. Measles matters, but do parents know. *Br Med J* 1985;**290:**623–4.

[14] Adjaye N, Azad A, Foster M, Marshall WC, Dunn H. Measles serology in children with a history of measles in early life. *Br Med J* 1983;**286:**1478.

[15] Hull D. Interpretation of the contra-indications to whooping cough vaccination. *Br Med J* 1981;**283**:1231–3.

[16] Wilkinson JR. Measles immunisation—contra-indications as interpreted by health visitors and clinic nurses. *Public Health* 1985;**99**:198–200.

[17] Lakhani ADH, Morris RW, Morgan M, Dale C, Vaile MSB. Measles immunisation: feasibility of a 90% target uptake. *Arch Dis Child* 1987;**62**:1209–4.

[18] Clarke SJ. Whooping cough vaccination: some reasons for non-completion. *J Adv Nurs* 1980;**5**:313–9.

[19] Colver AF. Immunisation strategy. *Lancet* 1987;**ii**:457.

[20] Noah ND. Immunisation before school entry: should there be a law? *Br Med J* 1987;**294**:1270–1.

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Sudden death in asthma

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SUMMARY Two girls with asthma died suddenly in autumn-winter 1985. On clinical criteria, both had been classified as moderately severe asthmatics, had shown a stabilised disease course, and had complied with antiasthmatic medication. By chance, lung function, bronchial reactivity, and subjective perception of lung function impairment had been assessed five and a half months and one month before death, respectively. For comparative purposes, we measured lung function and bronchial reactivity in a reference group of 37 children who had asthma of the same clinical severity and who were taking the same medication. In comparison with this reference group the index subjects showed a high degree of bronchial reactivity, incomplete recovery of function after bronchodilation, and appreciably reduced perception of severe lung function impairment. Regular assessment of lung function, bronchial reactivity, and perception of breathlessness might help to identify the patient who is prone to a fatal attack of asthma.

Childhood asthma is a major health problem, but updated concepts improve the therapeutic approach to the disease.[1 2] Nevertheless, there are still some children and adolescents who die of asthma, and many of these deaths occur suddenly.[3–8] While there are organised attempts to identify these patients,[9] such endeavours are hampered by the current lack of the objective data that are needed for identification of the subgroup at risk. There are only a few published lung function measurements from paediatric patients with asthma who later died suddenly[3 5]; so far, no observations on the bronchial reactivity of these patients have been reported.

We have previously studied the practical value of cold air challenge for measuring bronchial reactivity in children,[10 11] and now try to assess bronchial reactivity by this method once a year in all our patients with asthma of at least moderate severity. In autumn-winter 1985, two of our patients died suddenly at home, five and a half months and one month after this functional assessment, respectively. The present study reports measurements of lung function and bronchial reactivity in these two index patients and in a reference group with asthma of comparable severity.

Patients and methods

CASE 1

This girl died suddenly at the age of 14·8 years. She had been attending the local asthma clinic for the last two and a half years. Her case history showed extrinsic asthma beginning in the second year of life, three emergency hospital admissions, one hyposensitisation course with pollen antigen, and various courses of medication. In the year preceding death her asthma had appeared well stabilised by combined medication with an inhaled β_2 sympathomimetic, a topical steroid, and a sustained release theophylline preparation. She attended school regularly and was able to participate in various physical activities. On clinical criteria her asthma was graded to be of moderate severity (grade three on a scale from one to four); her compliance with medication was considered to be good. A one week peak flow protocol, recorded nine months before death, showed moderately wide diurnal variations and night dipping; subsequently her evening dose of the sustained release theophylline was increased, and two puffs of the bronchodilator aerosol were recommended, when waking up at night because of asthma symptoms.

Assessment of lung function and bronchial reactivity was performed five and a half months before death. In addition, the concentration of serum theophylline, and the presence of specific IgE antibodies to 12 common allergens were determined by fluorescence polarisation immunoassay and a radioallergosorbent assay (RAST), respectively (Abbott and Pharmacia Diagnostics). The concentration of serum theophylline was 84·4 µmol/l, and specific IgE to seven allergens was found positive.

The girl was last seen in the clinic two months before death. Her asthma appeared well stabilised, and spirometry showed moderate expiratory airflow obstruction; her medication regimen was not altered. Death occurred at home at 0·15 hours in a September night; there were no preceding symptoms of a viral infection, allergen exposure, or asthma exacerbation. The sister, sleeping in the same room, observed that the patient had become restless while still being asleep, then had got up from the bed and collapsed. Resuscitation efforts by family members were unsuccessful, and a local physician pronounced her dead 45 minutes later. In compliance with a request by the parents no postmortem examination was performed.

CASE 2

This girl died suddenly at the age of 9·4 years. She had attended the local asthma clinic for the previous four years. Her case history showed extrinsic asthma beginning in the fourth year of life and one emergency hospital admission. In the last year of life her asthma had appeared well stabilised by a combined medication with an inhaled β_2 sympathomimetic, a topical steroid, and a sustained release theophylline preparation. She lived with her divorced mother, attended school regularly, and was able to participate in physical activities at school and at a dancing class. On clinical criteria her asthma was graded to be of moderate severity (grade three on a scale from one to four), and her compliance with medication was considered to be satisfactory.

Assessment of lung function and bronchial reactivity was done one month before death. At this time the serum concentration of theophylline was 81·0 µmol/l; antibodies to six of 12 allergens were found by RAST. After this investigation she was instructed how to use a peak flow meter at home and was asked to record her peak expiratory flow rate twice a day and when having symptoms. This was, however, only done for the next five days and was subsequently neglected.

Death occurred at home around 7.00 hours on a November morning. The girl had suffered from a common cold for five days and had told her mother at 6.30 hours that she did not want to go to school because of feeling tired and short of breath. The mother found her lifeless on the floor of her room at about 7.15 hours. After emergency transport to a local hospital, she was pronounced dead on admission. Postmortem examination showed hyperinflated lungs with extensive mucus plugging of the peripheral airways.

REFERENCE GROUP

We recruited patients from the local asthma clinic, who were older than 8 years of age, and suffering from asthma of comparable clinical severity, and who were taking the same antiasthmatic medication as the index patients (β_2 sympathomimetic by metered dose inhaler, topical steroid, sustained release theophylline). This resulted in a reference group of 37 patients (28 boys, nine girls) with a mean age of 11·8 years (range 8·3 to 15·2).

ASSESSMENT OF LUNG FUNCTION AND BRONCHIAL REACTIVITY

Measurements in index and reference group patients were part of a routine programme and followed the same protocol. Informed consent by patients and parents was a prerequisite for all measurements. Functional assessment was done in a stable clinical condition twice within 10 days. Patients arrived at 9.00 hours, rested for 30 minutes, and then had baseline lung function testing.

At the first visit patients then inhaled a sympathomimetic β_2 bronchodilator (salbutamol, 0·02 ml of the 0·5% solution per kg of body weight) from a jet type nebuliser (Inhalierboy). Fifteen minutes after bronchodilator inhalation lung function testing was repeated.

Before the second visit, the sustained release theophylline medication was withheld for four days and the sympathomimetic for 12 hours. After baseline lung function testing, patients underwent a standardised cold air challenge according to a method described previously,[10] administered by a commercially available heat exchanger (RHES, Jaeger), which was set to apply absolutely dry air with a temperature of −10°C. Each challenge consisted of four minutes of isocapnic hyperventilation at 75% of maximal voluntary ventilation. The response was assessed by lung function testing three minutes after the termination of the cold air challenge. In addition, the patients were asked before and after the cold air challenge to score their subjective feeling of chest tightness as 'none', 'mild', 'moderate', or 'severe'.

Pulmonary function tests for baseline determinations and for measuring the responses were done on a pneumotachygraph spirometer (Pneumotest Junior, Jaeger). The patients performed several forced vital capacity manoeuvres, which were recorded in form of a maximum expiratory volume time and flow volume curve. Forced expiratory volume in the first second (FEV_1) and maximum expiratory flow at 25% remaining vital capacity ($\dot{V}_{max25}$) were measured and expressed as percent of predicted normal values.[12 13]

Thus the investigation resulted in two baselines, Table *Lung function and bronchial reactivity measurements. Results are % predicted*

	Case 1		*Case 2*		*Reference group (n=37)*			
	FEV_1	*$\dot{V}_{max25}$*	*FEV_1*	*$\dot{V}_{max25}$*	*FEV_1*		*$\dot{V}_{max25}$*	
					Mean (SD)	*Range*	*Mean (SD)*	*Range*
Baseline 1	84	55	35	22	89 (12)	40–111	64 (22)	16–115
Baseline 2	52	20	26	16	85 (15)	38–120	56 (23)	18–97
Functional optimum	91	67	82	61	96 (11)	73–126	84 (26)	31–151
Functional minimum	34	12	27	13	61 (20)	16–95	30 (17)	3–67
Range of reactivity	57	55	55	48	35 (18)	6–72	55 (24)	15–107

one measurement after the bronchodilator and one after the cold air challenge. These measurements were then interpreted according to a previously described concept of 'functional dimensions'.[11] Dimension one is baseline lung function. The second dimension is the level of function after bronchodilator medication, termed 'functional optimum'. Dimension three is a 'functional minimum', defined as a reproducible minimum function at maximum response to a standardised cold air challenge. The 'range of reactivity' is the distance from optimum to minimum.

Furthermore a 'perception score' was calculated for the pulmonary function tests before and after cold air challenge by multiplying the baseline and the post cold air challenge measurements (in percent predicted) with the subjective perception. For this purpose, a value of 1 was assigned to 'no', 2 to 'mild', 3 to 'moderate', and 4 to 'severe chest tightness'. Thus a low perception score stems from the combination of a low lung function measurement and underperception.

Results

Lung function from index patients and the reference group are compared in the table. Baseline function was better at the first visit—that is, on full treatment—than at the second visit—that is, without preceding bronchodilator medication. The second baseline measurements of case 1 were in the lower part and those of case 2 below the reference range. After the β_2 bronchodilator (functional optimum), both index patients normalised their FEV_1, but failed to completely normalise their $\dot{V}_{max25}$; thus they compared with the lower half of the reference range. After cold air challenge (functional minimum), both index patients showed a pronounced further increase of expiratory airflow obstruction and thus compared with the lower end of the reference range. The resulting range of reactivity (distance optimum to minimum) of the two index patients was in the middle of the reference range for $\dot{V}_{max25}$, and in the upper part of the reference range for FEV_1. For all lung function situations assessed, both index patients as well as the reference group showed a tendency towards more obstruction in $\dot{V}_{max25}$, than in FEV_1.

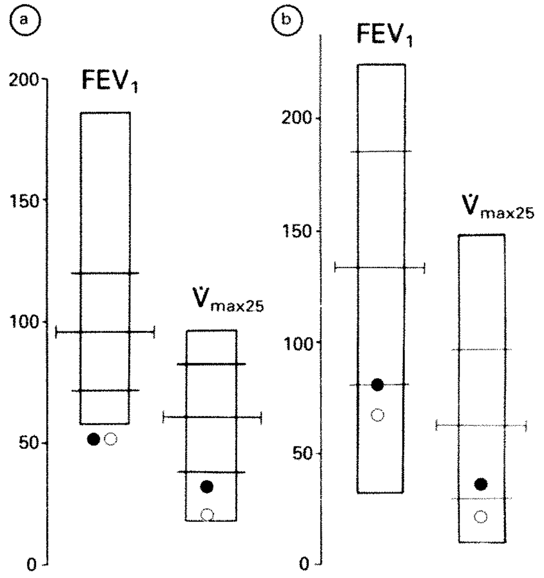

Figure *Perception score. The whole range of reference group values is given by the vertical bars; mean (SD) is indicated. Case 1=○; case 2=●. (a) Second baseline measurement (before cold air challenge); (b) minimum function measurement (after cold air challenge).*

The perception score assessed at baseline and the functional minimum is shown in the figure. Both index patients were below or at the lower end of the reference range for both functions.

Discussion

This study shows that two patients, who subsequently died suddenly, when compared with a reference group with asthma of identical clinical severity and medication, were characterised by an average or above average range of reactivity, which, however, was associated with a lower level of lung function. This was expressed by a tendency towards impaired baseline lung function, by incomplete normalisation after bronchodilator medication, and by severely compromised lung function after cold air challenge. Furthermore, both index patients showed a reduced perception of lung function impairment.

The case histories of the two index patients appear similar to previous descriptions of sudden asthma deaths.[3-5 7 8 14-16] Most likely, these sudden and fatal attacks are caused by a severe narrowing of intrathoracic airways; postmortem findings are characterised by mucus plugging of the peripheral bronchi.[3-5 14 17] Sudden death should be distinguished from other forms of asthma mortality, which might be caused by: (a) pulmonary or cardiac disorders or complications that went undetected before death; (b) toxic side effects of excessively overused antiasthmatic medication; and (c) prolonged exacerbations (status asthmaticus) resulting in death despite some therapeutic efforts.[5 6 8 16 18] Unsuspected pathology, especially tension pneumothorax, is unlikely in our two patients, but cannot be definitely exluded for case 1. Abuse of adrenergic aerosols can be excluded for causing the death of case 1, and remains a somewhat unlikely causative factor for the death of case 2. It therefore appears reasonable to assume that both index cases are true examples of 'sudden death in asthma'.

Anyone who attempts to reduce the mortality from such sudden and potentially fatal attacks faces the problem of identifying the patient at risk.[9] This task is related to the more general question of how to assess the severity of a disorder that is characterised by large variations in functional impairment.[19] Both index patients in the present study showed a functional profile of high bronchial reactivity in addition to impaired baseline function. Any obstructive airway reaction will sooner become life threatening, if it starts from an already compromised lung function level. Lung function and airway reactivity measurements in two patients do not suffice for a general explanation of sudden death in asthma: nevertheless, our observations suggest, that repeated assessments of bronchial reactivity might be helpful for identification of the fatality prone patient.

In many cases of fatal asthma the life threatening character of the disorder had not been perceived or acknowledged by patients, parents, or physicians.[4-7 15 16 18 20] Correspondingly, the present study suggests that an appreciable subjective underperception of lung function impairment could be an important risk factor. Previous studies on adult asthma patients have shown wide interindividual variations in the perception of airway obstruction; furthermore, blunted perception tends to correlate with high bronchial reactivity and thus will occur in more severely diseased patients.[21 22] Consequently, different longitudinal courses of variable airway obstruction could translate through different perception into similar case histories. This is illustrated by the present study, where all patients of the reference group had the same clinical severity grading but showed wide interindividual variations in their functional dimensions. The resulting clinical problem is how to identify those severely diseased patients who have adapted their life style to variable airway obstruction and therefore present with a seemingly benign case history.[19] Occasional lung function measurements cannot be used as a reliable yardstick for assessing disease severity, as lung function may change from hour to hour, day to day, or week to week.[19] Longitudinal peak flow recordings might be more meaningful for assessing the severity of a variable disorder and furthermore, they may gradually lead the patient towards a more accurate self assessment.[7 14 20] Peak flow recordings done during the day, however, might miss nocturnal deteriorations in lung function; this 'night dipping' might have some relevance for asthma mortality.[4 6 14]

Another potentially promising approach is suggested by the present study. Repeated assessments of lung function and bronchial reactivity could be complemented by the patient's self assessment of airway obstruction. Objectively measured physiological data, related to the patient's subjective score, could identify the 'poor perceivers' among the paediatric asthma population. This subgroup could then receive special diagnostic and therapeutic attention and a close longitudinal monitoring of clinical and physiological variables. Whether the combination of appreciable airway hyper-reactivity and severe underperception of airflow obstruction does indicate the necessity for a treatment with systemic steroids remains speculative. Other authors suggested that the therapeutic use of systemic steroids could have prevented some sudden deaths.[3-6 14] At present, any comparative discussion of different treatment strategies for the fatality prone asthma patient remains a speculative one.

The present study employed FEV_1 and end expiratory flow for characterising large and small airway obstruction respectively.[23] In both index patients and in the reference group as well, $\dot{V}_{max25}$ was found more compromised than FEV_1. Furthermore, in neither of the index patients was $\dot{V}_{max25}$ normalised after the bronchodilator. Correspondingly, another report on bronchodilator induced lung function changes in children who later died of asthma showed incomplete or absent responses for forced mid expiratory flow rates.[5] These observations correlate with the concept of childhood asthma as a disease of the small airways.[24 25] Inclusion of small airway related lung function measurements into the evaluation of paediatric asthma patients might be useful for better characterising disease severity.

In conclusion, we observed pronounced airway hyper-reactivity and severe underperception of airflow obstruction in two children with asthma who later died suddenly. These two observations do not suffice for a general explanation of sudden death in childhood and adolescent asthma. They do, however, suggest that repeated assessments of airway reactivity, complemented by the patient's subjective scoring of breathlessness, might be a promising approach towards better defining those children prone to sudden death from asthma.

References

[1] Pearlman DS. Bronchial asthma. A perspective from childhood to adulthood. *Am J Dis Child* 1984;**138**:459–66.

[2] König P. Asthma: a pediatric pulmonary disease and a changing concept. *Pediatr Pulmonol* 1987;**3**:264–75.

[3] Mellis CM, Phelan PD. Asthma deaths in children – a continuing problem. *Thorax* 1977;**32**:29–34.

[4] Rubinstein S, Hindi RD, Moss RB, Blessing-Moore J, Lewiston NJ. Sudden death in adolescent asthma. *Ann Allergy* 1984;**53**: 311–8.

[5] Kravis LP, Kolski GB. Unexpected death in childhood asthma. A review of 13 deaths in ambulatory patients. *Am J Dis Child* 1985;**139**:558–63.

[6] Carswell F. Thirty deaths from asthma. *Arch Dis Child* 1985;**60**: 25–8.

[7] Sears MR, Rea HH, Fenwick J, *et al.* Deaths from asthma in New Zealand. *Arch Dis Child* 1986;**61**:6–10.

[8] Kravis LP. An analysis of fifteen childhood asthma fatalities. *J Allergy Clin Immunol* 1987;**80**:467–72.

[9] Strunk RC. Workshop on the identification of the fatality-prone patient with asthma: summary of workshop discussion. *J Allergy Clin Immunol* 1987;**80**:455–7.

[10] Zach MS, Polgar G, Kump H, Kroisel P. Cold air challenge of airway hyperreactivity in children: practical application and theoretical aspects. *Pediatr Res* 1984;**18**:469–78.

[11] Zach MS, Polgar G, eds. Cold air challenge of airway hyperreactivity in children: dose-response interrelation with a reaction plateau. *J Allergy Clin Immunol* 1987;**80**:9–17.

[12] Polgar G, Promadhat V. *Pulmonary function testing in children: techniques and standards*. Philadelphia: WB Saunders, 1971:186.

[13] Zapletal A, Samanek M, Paul T. Upstream and total airway conductance in children and adolescents. *Bull Eur Physiopathol Respir* 1982;**18**:31–7.

[14] Bateman JRM, Clarke SW. Sudden death in asthma. *Thorax* 1979;**34**:40–4.

[15] Stableforth D. Death from asthma. *Thorax* 1983;**38**:801–5.

[16] Benatar SR. Fatal asthma. *N Eng J Med* 1986;**314**:423–8.

[17] Reid LM. The presence or absence of bronchial mucus in fatal asthma. *J Allergy Clin Immunol* 1987;**80**:415–6.

[18] Nguyen MT, Patterson K, Sly RM. Causes of death from asthma in children. *Ann Allergy* 1985;**55**:448–53.

[19] McNicol KN, Williams HB. Spectrum of asthma in children – I. clinical and physiological components. *Br Med J* 1973;**iv**:7–11.

[20] Cushley MJ, Tattersfield AE. Sudden death in asthma: discussion paper. *J R Soc Med* 1983;**76**:662–6.

[21] Rubinfeld AR, Pain MCF. Perception of asthma. *Lancet* 1976;**i**: 882–4.

[22] Burdon GW, Juniper EF, Killian KJ, Hargreave FE, Campbell EJM. The perception of breathlessness in asthma. *Am Rev Respir Dis* 1982;**126**:825–8.

[23] Hyatt RE, Rodarte JR, Mead J, Wilson TA. Changes in lung mechanics: flow-volume relations. In: Macklem PT, Permutt S, eds. *The lung in the transition between health and disease*. New York: Marcel Dekker, 1979:73–112.

[24] Zapletal A, Motoyama EK, Gibson LE, Bouhuys A. Pulmonary mechanics in asthma and cystic fibrosis. *Pediatrics* 1971;**48**: 64–72.

[25] Hill DJ, Landau LJ, McNicol KN, Phelan PD. Asthma – the physiological and clinical spectrum in childhood: respiratory function studies in its assessment. *Arch Dis Child* 1972;**47**: 874–81.

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Commentary

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Much has been written about the shortcomings in the care of asthmatics who have died in this country. The British Thoracic Association's review identified only 10 of 90 asthmatics in whom death was considered unavoidable.[1] It would be impossible, however, to test whether management is more deficient in those who die than in asthmatics in general because of the difficulty in recruiting controls from the same practice. Carswell, in a review of 30 deaths in children, concluded that inadequate management had probably contributed to many of these deaths.[2] Twelve of the 30 children had never attended hospital. Speight and colleagues in 1983 highlighted the problem of underdiagnosis and undertreatment of childhood asthma[3]; one wonders what overall effect educational programmes have since had in addressing this very serious problem. Two American studies of asthma deaths in children drew attention to the unsatisfactory psychosocial profiles of many of the patients and their families, which resulted in erratic treatment.[4 5] It is difficult to compare the American experience with that in Britain because of the differences in the delivery of health care, but it is nevertheless clear that most children die in circumstances where there has been poor medical or parental supervision, or both.

The two children reported here were considered to have moderately severe asthma. The first, who died during the night, was known to suffer from night time wheezing; that is an extremely worrying feature. The second was known to have been absent from school in the year before her death and had had a cold in the previous five days. It is not mentioned whether adequate bronchodilators had been given during this time. The results of bronchial challenge testing in these two girls suggested that they were different from a group of children who also had moderately severe asthma and thus the two may have been identified as 'fatality prone'. These girls clearly had 'clinically labile' asthma but by implication no different from the others in the group.

Perhaps the classification of asthmatics into groups according to severity is no longer very helpful. If children miss school because of asthma or need emergency treatment then their asthma is troublesome and thus needs further attention. If exercise induced wheezing or night time waking are problems then treatment needs to be directed towards control of this clinical evidence of bronchial reactivity. Those caring for asthmatic children—parents, physicians, teachers, and so on—need to know what triggers wheezing (for example, upper respiratory tract infections, parties, grandma's cat, school examinations) and need to know how to take effective steps to prevent it. The understanding and use of the home peak flow gauge will help identify abnormal diurnal variations in peak expiratory flow rate and draw attention to those children who are slow to perceive bronchoconstriction. The parents of every child who can use a peak flow gauge properly should know what his or her best possible measurement is—*not* necessarily the same as the mean value read off the chart that comes with the instrument (a common misunderstanding)—so that falls in readings can be identified early and acted upon. Most children can be taught to use a gauge properly before they start school, although they may not understand the significance of the readings. When children are known not to be receiving adequate parental supervision of their asthma, teachers should be encouraged to administer all drugs if necessary.

Once asthmatics are clinically stable and they and their families understand their disorder, and know what to do to prevent attacks, then it would be interesting to know if there are differences between them in the results of detailed lung function and bronchial challenge testing. It would also be interesting to know whether with intensive education perception of airway narrowing improves. If differences exist then a group can be singled out for further attention. With optimum clinical control morbidity would certainly be reduced but it would have to be seen if this was reflected in a drop in mortality. There will still remain a small group of very worrying asthmatics who despite all precautions, therapeutic and otherwise, will be at risk of sudden severe attacks. These patients and their families need to be drilled in emergency measures so that they are able to reach hospital before irreversible damage is done.

References

[1] Research Committee of the British Thoracic Association. Death from asthma in two regions of England. *Br Med J* 1982;**285:** 1251–5.

[2] Carswell F. Thirty deaths from asthma. *Arch Dis Child* 1985;**60:** 25–8.

[3] Speight ANP, Lee DA, Hey EN. Underdiagnosis and undertreatment of asthma in childhood. *Br Med J* 1983;**286:**1253–6.

[4] Kravis LP. An analysis of fifteen childhood asthma fatalities. *J Allergy Clin Immunol* 1987;**80:**467–72.

[5] Strunk RC. Asthma deaths in childhood: identification of patients at risk and intervention. *J Allergy Clin Immunol* 1987; **80:**472–5.

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Changes in asthma prevalence: two surveys 15 years apart

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SUMMARY In 1973 a survey was conducted among 12 year old children living in a defined area of South Wales. In 1988 the survey was repeated in the same area, again among 12 year old children. Questionnaires were completed for all 965 children in the population sample; peak expiratory flow rates were performed on them all, and repeated (except for five children) after an exercise provocation test. The prevalence of a history of wheeze at any time had increased from 17% to 22%, while that of a history of asthma at any time had increased from 6% to 12%. Current asthma had increased from 4% to 9%, but wheezing in the past year not attributed to asthma had remained at 6%. The exercise provocation tests suggested that both mild and severe asthma had become more common. Increases had also occurred in the frequencies of a history of eczema (from 5% to 16%) and of hay fever (from 9% to 15%). It seems that the prevalence of asthma has risen, and that this cannot be wholly explained by a greater readiness to diagnose the disease.

There is some dispute as to whether the prevalence of childhood asthma is rising in Britain.[1-3] A phenomenal rise has occurred in hospital admission rates,[3] but this may be attributable to changes in admission policy. The consulting rates for patients attending general practitioners for asthma have also increased,[4] but this may merely reflect a greater readiness to diagnose asthma in wheezing children. In the absence of serial surveys conducted in the same areas with the same methodology it is not clear whether the prevalence is really changing. This paper describes a survey of 12 year old school children living in the same areas of South Wales in which a similar survey was conducted 15 years before, using the same methodology—that is, questionnaires and exercise provocation tests.

Subjects and methods

The survey was first conducted in 1973 and the findings then have already been published.[5] It was repeated in 1988 in the same schools as before, together with another school that had opened in one of the catchment areas during the interval. As before, children who lived in the defined catchment areas and attended state supported Anglican, Roman Catholic, or Welsh schools were also included. The population sample comprised the children in their first year of high school, their 12th birthdays falling during the current school year.

A short questionnaire was sent to the parents containing the same questions as those in the 1973 questionnaire. The children were seen in groups at their schools and the peak expiratory flow rates (PEFR) and heights measured. Exercise testing was carried out at the same time of year (April-July) and in the same way as in 1973, with two minor modifications. Firstly, any child who was unable to attain 70% of the PEFR predicted from the 1973 data (allowing for height) was not exercised then but asked to attend on another occasion in order to avoid provocation testing when bronchoconstriction was already present. Secondly, children who were currently taking treatment for asthma were exercised with the others; if their PEFRs did not fall by more than 15% they were asked to return for another exercise test when they had not taken treatment other than steroids (oral or inhaled) for at least eight hours. If the PEFR fell by 15% or more when the child had taken treatment within eight hours the exercise test was not repeated; this was in case exercise provocation in the absence of treatment produced severe bronchoconstriction. In 1973 all children who had taken treatment within five hours of the test were re-exercised on another occasion when they had not taken recent treatment, whatever the original results. The exercise test consisted of free running for six minutes in the school hall or gymnasium, followed by five minutes resting. Five PEFR values were recorded immediately before the exercise and at the end of the rest period, the mean of the three highest readings being taken as the true value on each occasion.

Results

A total of 965 children (495 boys, 470 girls) were identified as eligible for the survey. The number was higher than that in 1973 (818) owing to residential developments and some changes in catchment areas of the schools. A questionnaire was completed for every child. Table 1 shows the percentages of children for whom various conditions were reported; a few errors in the 1973 data have been corrected, causing slight differences from the previously published figures. 'Current asthma' denotes the children (51 boys and 37 girls) who were said to have had asthma and to have wheezed during the past 12 months. The proportion reporting a history of asthma (currently or at any time) had doubled from the first to the second survey. The percentage reporting wheezing had also risen, but this increase was accounted for by the higher prevalence of asthma, the proportion with wheeze in the past 12 months but no asthma being about the same (6%) on each occasion. The prevalence of a history of eczema had risen to an even greater extent (from 5% in 1973 to 16% in 1988), while hay fever had risen from 9% to 15%.

Table 2 relates to those subjects who had ever wheezed. Certain factors were listed on the questionnaire as possible causes of wheezing. More than one factor could be selected, so the numbers are not mutually exclusive. There was a substantial rise in the prevalence of wheezing attributed to running, to contact with animals, and to food, and the proportion of wheezy children whose symptoms were attributed to these factors also increased. There was less change in the percentages attributing wheezing to worry or excitement; wheezy colds had a similar prevalence in the two surveys but declined as a proportion of all wheezy children.

Table 2 *Attributed causes of wheezing in the two surveys*

Attributed cause	*1973 Survey*			*1988 Survey*		
	No	*% Of all children (n=817)*	*% Of wheezers (n=138)*	*No*	*% Of all children (n=965)*	*% Of wheezers (n=215)*
Colds	125	15·3	90·6	166	17·2	77·2
Running	47	5·8	34·1	101	10·5	47·0
Worry	22	2·7	15·9	27	2·8	12·6
Excitement	27	3·3	19·6	44	4·6	20·5
Animals	14	1·7	10·1	35	3·6	16·3
Foods	1	0·1	0·7	16	1·7	7·4

Table 3 *Changes in PEFR on exercise: cumulative percentages of children with (PEFR2/PEFR1) × 100 below various levels*

(PEFR2/PEFR1) × 100 (%)	*1973 Survey*		*1988 Survey*	
	Cumulative %	*No*	*Cumulative %*	*No*
<25	—	0	0·2	2
<35	0·1	1	0·3	3
<45	0·2	2	0·8	8
<55	0·4	3	1·3	12
<65	0·9	7	2·3	22
<75	2·0	16	4·1	39
<85	6·7	54	7·7	74
<95	36·7	298	30·8	296
<105	91·4	742	89·0	854
<115	99·1	805	99·6	956
<125	100	812	99·9	959
<135	100	812	100	960

Initial PEFR and height were measured in all 965

Table 1 *Prevalence of history of symptoms in two surveys. Results are %*

Condition	*1973 Survey*			*1988 Survey*			*1988–1973 Difference (95% confidence interval)*
	Boys (n=413)*	*Girls (n=405)*	*All* (n=818)*	*Boys (n=495)*	*Girls (n=470)*	*All (n=965)*	
Asthma ever	7·0	4·0	5·5	14·1	9·8	12·0	6·5 (3·9 to 9·1)
Current asthma	5·6	2·7	4·2	10·3	7·9	9·1	5·0 (2·7 to 7·2)
Wheeze ever	21·3	12·6	17·0	26·7	17·7	22·3	5·3 (1·6 to 9·0)
Wheeze in past 12 months	12·3	7·2	9·8	17·8	12·6	15·2	5·5 (2·4 to 8·5)
Breathless wheeze ever	11·7	6·7	9·2	17·2	10·6	14·0	4·8 (1·9 to 7·8)
Wheeze without cold ever	8·7	4·4	6·6	16·4	11·1	13·8	7·2 (4·4 to 9·9)
Eczema ever	5·6	4·0	4·8	16·0	15·7	15·9	11·1 (8·4 to 13·8)
Hay fever ever	11·2	7·7	9·4	18·4	11·3	14·9	5·5 (2·5 to 8·5)

*Includes one boy with current asthma about whom no other information was obtained.

children. The mean (SD) PEFR was 328 (58) l/minute in the boys with current asthma and 350 (48) l/minute in the other boys, the mean values for girls being 332 (58) and 351 (51) l/minute respectively. The difference in boys was significant ($p<0{\cdot}01$) whereas in girls it just failed to reach significance ($t=1{\cdot}94$) using unpaired t tests. Mean heights were 1470 (64) and 1491 (71) mm for the asthmatic and non-asthmatic boys respectively, and 1498 (78) and 1509 (73) mm respectively for the asthmatic and non-asthmatic girls. When corrected for height (using simple linear regression), mean PEFR was 5% lower in the boys with asthma than in those without ($p<0{\cdot}05$), and 4% lower in the girls with asthma than in those without, but this was not significant.

Five children did not exercise because of conditions other than asthma: one had currently treated asthma, one had wheezed during the past year, one had wheezed before the past year, and two had never wheezed. Four children could not attain 70% of the predicted PEFR, so they were recalled and exercised on a further occasion when their PEFRs were higher. Of 39 children who had taken bronchodilators, cromoglycate, or inhaled steroids during the eight hours before the exercise test, 16 re-exercised without treatment, three re-exercised having taken inhaled steroids only, and 20 did not re-exercise as their PEFRs fell by at least 15% on the first occasion. In the 1973 survey six children re-exercised because they had taken medication for asthma within the past five hours. In five of these cases the original PEFRs fell by more than 15% on exercise, so the results of the first exercise tests were used in comparisons with the 1988 survey as they would not have been re-tested had they been seen in 1988. Two other children in 1973 had taken cromoglycate during the eight hours before the test and were not retested; in one of these the PEFR fell

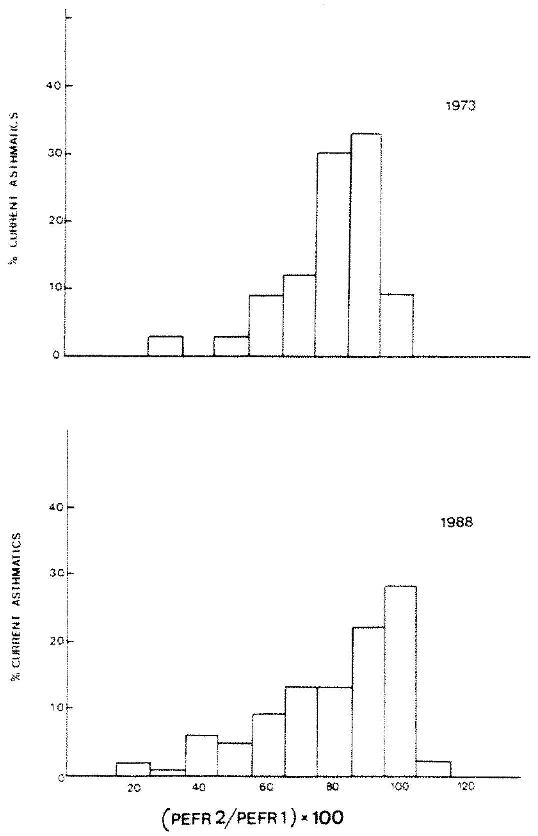

Fig 1 *Effect of exercise on children with current asthma.*

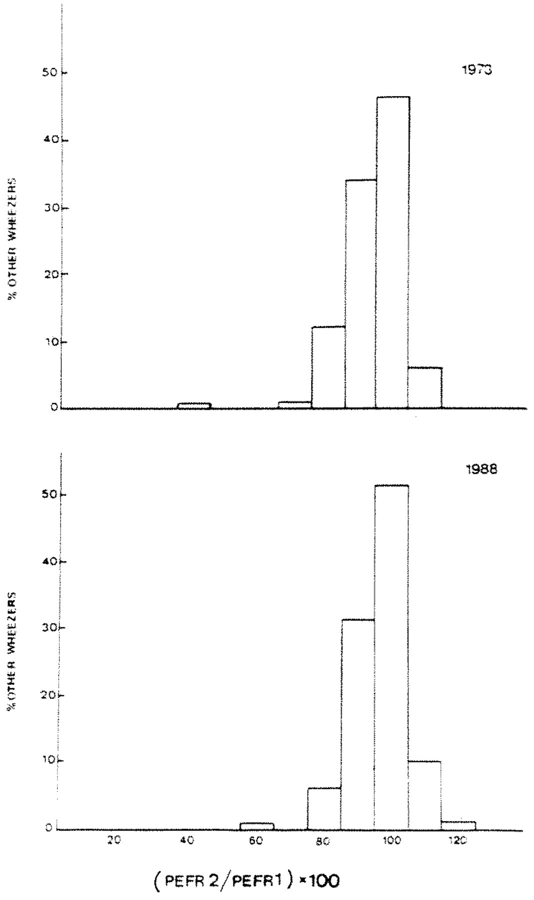

Fig 2 *Effect of exercise on other children who had ever wheezed.*

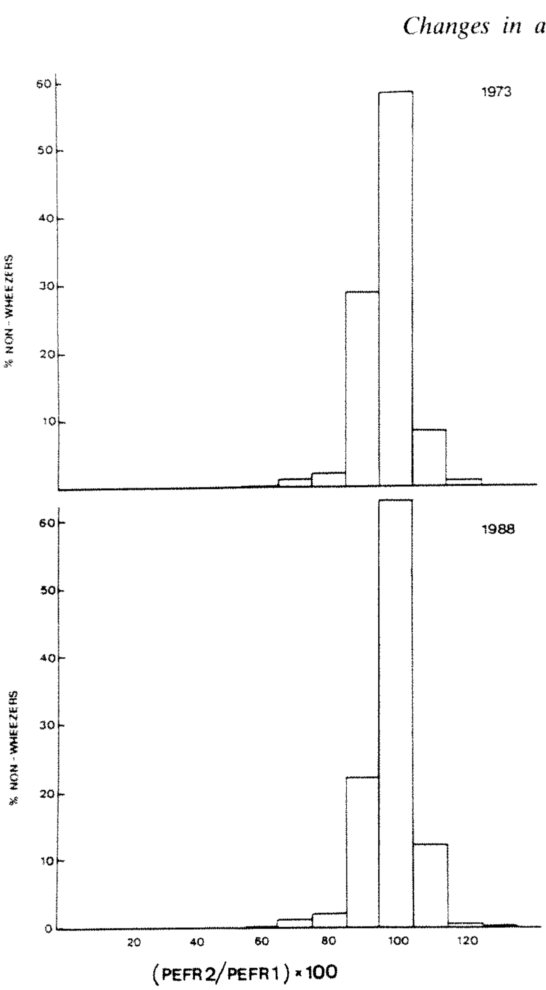

Fig 3 *Effect of exercise on children who had never wheezed.*

by more than 15% so the test would not have been repeated in 1988. Six did not exercise in 1973.

The PEFR measured after exercise was expressed as a percentage of the initial PEFR for each child: (PEFR2/PEFR1) × 100. In both surveys there was no significant sex difference in the mean (PEFR2/PEFR1) × 100 among asthmatic or non-asthmatic children, so the data from boys and girls were combined. Table 3 shows cumulative percentages of the children in the two surveys with (PEFR2/PEFR1) × 100 values below various levels. A low value occurred more often in 1988 than in 1973 ($p<0·05$): a value of 75% or less occurred twice as frequently in the second survey than in the first, and lower values showed an even greater disparity. In the middle and upper parts of the distribution the differences were less consistent.

Fig 1 shows the distribution of (PEFR2/PEFR1) × 100 in the children with current asthma in the two surveys (33 in 1973, 87 in 1988). On each occasion there was a considerable skew to the left, and in 1988 the values were distributed more widely than in 1973. The mean value was very similar on the two occasions (80·8 in 1973 and 79·9 in 1988), but the variance was significantly greater ($p<0·01$) in the second survey, the SD being 14·2 and 21·4 respectively.

Fig 2 shows the distributions of (PEFR2/PEFR1) × 100 in the other children who had ever wheezed (105 in 1973, 125 in 1988). Here the 1988 distribution was somewhat more compact than that of 1973, with less of a leftward skew. The mean (SD) value was 93·5 (9·2) in 1973 and 96·2 (7·6) in 1988, the difference in variance being significant at $p<0·05$. Data from the children who had never wheezed (674 in 1973, 748 in 1988) are shown in fig 3. The distributions showed little skew and were not dissimilar, but there was some tendency for values to be higher in the second survey; the mean values were 97·2 (6·5) and 98·3 (6·5) respectively.

Discussion

This survey was undertaken primarily to see whether the prevalence of asthma had changed since a similar survey 15 years ago. In 1973 only one child (known to have asthma) failed to participate, and in 1988 the response rate to the questionnaire was 100%, so selective bias was avoided. The same investigator conducted both surveys, using exactly the same procedures except for slight modifications that involved very few children. It is therefore most unlikely that the differences in the findings could be attributable to any changes of methodology.

In several countries there has been some anxiety about a possible rise in asthma mortality during recent years, particularly among young people.[6–9] The issue is complicated by changes in diagnostic practice and coding rules, and there is some dispute about the underlying trend.[1–3] It is difficult to obtain accurate information about changes in the prevalence of asthma owing to the lack of a suitable definition. In the present study no attempt was made to define asthma; the prevalence of reported asthma and wheezing was compared on two occasions together with the response to a simple exercise challenge test.

The results are in accord with the reported increase in the number of people consulting general practitioners for asthma.[4] The rise in asthma prevalence cannot be attributed solely to a change in diagnostic terminology, as the prevalence of wheezing not diagnosed as asthma has remained constant. As a further attempt to distinguish a genuine increase from a greater readiness to diagnose the disease, questions were asked about symptom patterns suggestive of asthma (wheezing with breathlessness in the absence of a cold, on running, or on contact with animals) without using the word 'asthma'. In each case the prevalence increased appreciably between 1973 and 1988. Among children who had ever wheezed, the proportion whose wheezing was attributed to running or animals had increased, again suggesting a real rise in asthma prevalence rather than better reporting of wheeze; the increased attribution to foods may perhaps simply reflect greater publicity given to food allergy now than in 1973. A fourfold rise occurred of children who had taken bronchodilators or other treatment for asthma within the eight hours before the interview, from eight (1%) in 1973 to 39 (4%) in 1988. This confirmed the impression of several of the school teachers, who remarked that the number of children taking treatment for asthma had greatly increased during the last few years.

There had also been an appreciable increase in children with histories of eczema (threefold) and hay fever (by about 50%). Other studies have reported similar increases in these conditions during recent years.[4] [10] It is of course accepted that the actual figures are very imprecise, owing to the vagaries of memory and diagnostic fashion. But it seems very unlikely that the reporting of all three conditions would have increased to this extent and with this degree of consistency unless allergic disease was really becoming more common.

The exercise challenge provided some objective evidence in support of the questionnaire findings. Although a fall in PEFR of 25% or more was uncommon, it occurred more frequently in 1988 than in 1973, and the increase in the more severe reactions was proportionately even greater. Children said to have current asthma had a somewhat wider distribution of (PEFR2/PEFR1) × 100 in 1988 than in 1973—that is, if the response to exercise indicates severity of asthma, both mild asthma and severe asthma had become more common. Among the other children with a history of wheeze the leftward skew in 1988 was less than that in 1973. Taken together these changes suggest that there has been both a real increase in the prevalence of asthma and a tendency to diagnose asthma in 1988 among wheezy children who would previously have been regarded as non-asthmatic. The children who had never wheezed showed a similar pattern on the two occasions, except that the mean PEFR% was slightly higher in 1988.

It is not at all clear what factors could have caused the prevalence of asthma to rise. The genetic composition of the populations surveyed appeared to be unchanged: the area contained very few people of immigrant stock on either occasion. Atmospheric pollution has been associated with a higher prevalence of asthma,[11] [12] but this is not an obvious problem in the area where this study was conducted. Studies of Xhosa children[13] and Tokelau islanders[14] suggest that the risk of asthma is associated with the modern western way of life. It may be that some environmental factor that provokes asthma has become more common during recent years. Alternatively a cohort effect has been postulated,[2] determined by factors operating near the time of birth. The identity of such factors can only be conjectured at present.

Similar surveys are being conducted in several other countries, and comparative findings will be presented in due course.

We are most grateful to the staff of the schools concerned in this survey for all their help and to the children for their participation.

References

1 Burr ML. Is asthma increasing? *J Epidemiol Community Health* 1987;**41**:185–9.

2 Burney P. Asthma deaths in England and Wales 1931–85: evidence for a true increase in asthma mortality. *J Epidemiol Community Health* 1988;**42**:316–20.

3 Anderson HR. Is the prevalence of asthma changing? *Arch Dis Child* 1989;**64**:172–5.

4 Fleming DM, Crombie DL. Prevalence of asthma and hay fever in England and Wales. *Br Med J* 1987;**294**:279–83.

5 Burr ML, Eldridge BA, Borysiewicz LK. Peak expiratory flow rates before and after exercise in school children. *Arch Dis Child* 1974;**49**:923–6.

6 Jackson RT, Beaglehole R, Rea HH, Sutherland DC. Mortality from asthma: a new epidemic in New Zealand. *Br Med J* 1982;**285**:771–4.

7 Stewart CJ, Nunn AJ. Are asthma mortality rates changing? *Br J Dis Chest* 1985;**79**:229–34.

8 Markham D, Chang M, Evans R, Mulally D. Epidemiologic study of deaths from asthma among children in US 1965–1983. *J Allergy Clin Immunol* 1986;**77**:161.

9 Burney PGJ. Asthma mortality in England and Wales: evidence for a further increase. *Lancet* 1986;**ii**:323–6.

10 Taylor B, Wadsworth J, Wadsworth M, Peckham C. Changes in the reported prevalence of childhood eczema since the 1939–45 war. *Lancet* 1984;**ii**:1255–7.

11 Andrae S, Axelson O, Bjorksten B, Fredriksson M, Kjellman N-IM. Symptoms of bronchial reactivity and asthma in relation to environmental factors. *Arch Dis Child* 1988;**63**:473–8.

12 Goren AI, Hellmann S. Prevalence of respiratory symptoms and diseases in school children living in a polluted and in a low polluted area in Israel. *Environ Res* 1988;**45**:28–37.

13 Van Niekerk CH, Weinberg EG, Short SC, Heese HdeV, van Schalkwyk DJ. Prevalence of asthma: a comparative study of urban and rural Xhosa children. *Clin Allergy* 1979;**9**:319–24.

14 Waite DA, Eyles EF, Tonkin SL, O'Donnell TV. Asthma prevalence in Tokelauan children in two environments. *Clin Allergy* 1980;**10**:71–5.

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Mortality from congenital malformations in England and Wales: variations by mother's country of birth

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SUMMARY Stillbirth and infant mortality from congenital malformations in England and Wales during 1981–5 was investigated according to the mother's country of birth. Significant differences remained after standardising for maternal age and social class. The highest overall mortality was in infants of mothers born in Pakistan (standardised mortality ratio 237), followed by infants of mothers born in India (standardised mortality ratio 134), East Africa (standardised mortality ratio 126), and Bangladesh (standardised mortality ratio 118). Caribbean and West African mothers showed an overall deficit. Mortality was inversely related to social class in all groups except the Afro-Caribbean. Infants of mothers born in Pakistan had the highest mortality in every social class except I, and for most anomalies investigated. Their ratios were particularly high for limb and musculoskeletal anomalies (standardised mortality ratio 362), genitourinary anomalies (standardised mortality ratio 268), and central nervous system anomalies (standardised mortality ratio 239). Our findings highlight the need for further research to identify the causes underlying these differences.

Ethnic differences in the incidence of congenital malformations have been recorded in many countries, including Britain.[1-13] Regional studies in this country have reported comparatively low rates of malformations among Afro-Caribbean subjects and comparatively high rates among Asians.[1-3] Differences in types of malformations and incidence patterns within the Asian community have been less well documented. A study of mortality from malformations in England and Wales for the years 1976–80 showed significant differences between immigrant groups, and within the different Asian groups.[4]

In this study we have investigated ethnic differences in mortality from malformations in England and Wales in greater detail for 1981–5, the latest years for which national data were available. Infants of mothers born in India, Pakistan, and Bangladesh were analysed separately, in addition to those born to mothers from East and West Africa, Republic of Ireland, and the Caribbean. Because mortality from congenital anomalies in England and Wales is associated with social class,[14] we have also investigated the contribution of social class to mortality within the different ethnic groups.

Methods

Mortality from congenital malformations in England and Wales during 1981–5 was analysed according to the mother's country of birth, using data from the Office of Population Censuses and Surveys (OPCS). Stillbirths and infant deaths with congenital anomalies as the underlying cause of death were examined for mothers born in the United Kingdom, Republic of Ireland, India, Bangladesh, East and West Africa, Pakistan, and the Caribbean. For convenience, mothers born in the Indian subcontinent are sometimes referred to as Asians.

Although information on ethnic origin is not directly available, the mother's country of birth is a reasonable substitute for ethnicity for most women of childbearing ages.[15] The only notable exceptions are East African immigrants, most of whom are of Indian origin.[16 17] The groups studied do not, however, include second generation immigrant mothers, who have been classified as born in the United Kingdom. Their numbers are too small to have a significant effect on the results for mothers born in the United Kingdom.[18]

OPCS codes a 10% sample of live births for social

Table 1 *Mortality from congenital malformations as a proportion of births and infant deaths, by mother's country of birth, England and Wales: 1981–5*

Mother's country of birth	*Total No of live births and stillbirths*	*Total No of infant deaths and stillbirths from all causes*	*Infant deaths and stillbirths from congenital malformations*		
			Total No	*% Of all births*	*% Of infant deaths and stillbirths*
United Kingdom	2796404	43459	8895	0·32	20·5
Republic of Ireland	33941	603	127	0·37	21·1
India	58851	1071	268	0·46	25·0
Bangladesh	18837	359	92	0·49	25·6
East Africa	35156	606	140	0·40	23·1
West Africa	14112	254	37	0·26	14·6
Caribbean	27612	546	91	0·33	16·7
Pakistan	67065	1846	601	0·90	32·6
Total births: England and Wales	3202958	50869	10647	0·33	20·9

class. Social class (derived from father's occupation) is given for legitimate births and illegitimate births registered by both parents. Deaths among illegitimate births registered by the mother alone are coded separately. The social class groups studied were: I professional and managerial, II intermediate, IIIn skilled non-manual, IIIm skilled manual, IV partly skilled, and V unskilled. For ease of presentation, the social classes were aggregated into non-manual (I–IIIn) and manual (IIIm–V).

Ethnic differences in mortality from all malformations and selected anomalies were investigated using standardised mortality ratios. The ratios were standardised for maternal age and social class with rates for England and Wales as standard. Age standardised mortality ratios were also examined for ethnic differences in mortality within social class groups. The significance of differences between groups was assessed by χ^2 test and a p value of $<0·05$ was accepted as significant; 95% confidence intervals (CI) are given for the principal comparisons.

Results

During 1981–5 there were 10 647 stillbirths and infant deaths from congenital abnormalities in England and Wales, constituting 0·33% of all births during the same period (table 1). This proportion was generally high in groups of Asian origin, being highest in Pakistanis (0·90%). Malformation deaths as a proportion of all infant deaths and stillbirths were comparatively low in West Africans and Caribbeans, and highest in Pakistanis. Types and patterns of mortality from congenital malformations differed significantly among immigrant communities, irrespective of maternal age and social class. Overall mortality from malformations was low in

Table 2 *Standardised mortality ratios and numbers of infant deaths and stillbirths for all congenital anomalies, by mother's country of birth, England and Wales: 1981–5*

Mother's country of birth	*All congenital anomalies*		
	Mortality ratio standardised for mother's age and social class (England and Wales=100)	*Observed deaths*	*95% Confidence interval*
United Kingdom	96	8895	94 to 98
Republic of Ireland	107	127	89 to 126
India	134	268	118 to 150
Bangladesh	118	92	96 to 145
East Africa	126	140	105 to 147
West Africa	74	37	53 to 102
Caribbean	93	91	76 to 114
Pakistan	237	601	218 to 256
All mothers: England and Wales	100	10647	

West Africans and Caribbeans (table 2). Infants of mothers born in India, East Africa, and Bangladesh showed moderate excesses. The highest mortality (standardised mortality ratio 237) was found in Pakistani infants.

In all groups except West Africans and Caribbeans mortality was higher in the manual than in the non-manual classes, with the groups of Asian origin showing a significant and consistent excess in the manual category (table 3). In the non-manual category only Pakistanis showed a significant excess (standardised mortality ratio 191). A detailed examination by social class showed that Pakistanis had the highest standardised mortality ratios in every social class except I (one death), with mortality in social classes II–V being 2·5–3·1 times the corresponding rates for mothers born in the United Kingdom. Although the other Asian groups also showed consistently raised mortality in social classes II–V, their rates were considerably lower than those for Pakistani infants.

Mortality from central nervous system anomalies (table 4) was highest in infants of mothers born in Pakistan (standardised mortality ratio 239), with Indians showing a smaller but nonetheless significant excess (standardised mortality ratio 142).

Table 3 *Standardised mortality ratios and numbers of infant deaths and stillbirths for all congenital anomalies, by mother's country of birth and social class, England and Wales: 1981–5*

Mother's country of birth	*Non-manual*		*Manual*		*Other*		*Sole registered illegitimate*		*Total*	
	Mortality ratio standardised for mother's age†	*Observed deaths*	*Mortality ratio standardised for mother's age†*	*Observed deaths*	*Mortality ratio standardised for mother's age†*	*Observed deaths*	*Mortality ratio standardised for mother's age†*	*Observed deaths*	*Mortality ratio standardised for mother's age†*	*Observed deaths*
United Kingdom	83*	2703	100	5077	134*	414	112*	701	96*	8895
Republic of Ireland	93	39	123	76	34	1	128	11	110	127
India	107	70	148*	183	233*	14	100	1	137*	268
Bangladesh	105	12	138*	77	242	3	0	0	134*	92
East Africa	117	70	131*	65	86	3	167	2	123*	140
West Africa	75	16	36	3	91	12	153	6	79	37
Caribbean	118	27	86	41	94	3	104	20	98	91
Pakistan	191*	79	272*	490	353*	29	455*	3	261*	601
All mothers: England and Wales	85*	3192	106*	6161	131*	534	113*	760	100	10647

*p<0·05; †England and Wales=100.

Table 4 *Standardised mortality ratios and numbers of infant deaths and stillbirths by mother's country of birth for anomalies of the central nervous system, England and Wales: 1981–5*

Mother's country of birth	*Anencephalus*		*Spina bifida*		*Other central nervous system anomalies*		*All central nervous system anomalies*	
	Mortality ratio standardised for mother's age and social class†	*Observed deaths*	*Mortality ratio standardised for mother's age and social class†*	*Observed deaths*	*Mortality ratio standardised for mother's age and social class†*	*Observed deaths*	*Mortality ratio standardised for mother's age and social class†*	*Observed deaths*
United Kingdom	95	663	104	1118	90*	770	97	2551
Republic of Ireland	160	14	113	14	113	12	126	40
India	149	22	97	23	196*	36	142*	81
Bangladesh	118	8	57	5	128	10	98	23
East Africa	113	9	103	13	185*	18	132	40
West Africa	90	3	20	1	113	5	71	9
Caribbean	54	4	10*	1	44	4	33*	9
Pakistan	268*	54	101	30	379*	94	239*	178
All mothers: England and Wales	100	803	100	1231	100	984	100	3018

*p<0·05; †England and Wales=100.

Table 5 *Standardised mortality ratios and numbers of infant deaths and stillbirths, by mother's country of birth, for selected anomalies, England and Wales: 1981–5*

Mother's country of birth	*Anomalies of circulatory system*		*Anomalies of respiratory system*		*Cleft lip/palate, anomalies of upper alimentary tract and digestive system*		*Anomalies of genitourinary system*		*Limb and musculoskeletal anomalies*		*Chromosomal anomalies*	
	Mortality ratio standardised for mother's age and social class†	*Observed deaths*	*Mortality ratio standardised for mother's age and social class†*	*Observed deaths*	*Mortality ratio standardised for mother's age and social class†*	*Observed deaths*	*Mortality ratio standardised for mother's age and social class†*	*Observed deaths*	*Mortality ratio standardised for mother's age and social class†*	*Observed deaths*	*Mortality ratio standardised for mother's age and social class†*	*Observed deaths*
United Kingdom	98	2837	98	651	97	363	98	462	94	703	97	426
Republic of Ireland	95	36	132	11	120	6	91	5	85	8	151	11
India	138*	86	76	11	136	12	112	12	88	14	84	8
Bangladesh	114	26	94	5	181	5	160	6	99	6	87	4
East Africa	132	47	75	6	84	4	116	7	103	9	140	8
West Africa	78	13	57	2	49	1	80	2	75	3	122	3
Caribbean	114	35	213*	15	76	3	86	4	128	10	114	6
Pakistan	169*	130	182*	33	224*	22	268*	35	362*	73	109	14
All mothers:												
England and Wales	100	3346	100	766	100	431	100	545	100	857	100	515

*p<0·05; †England and Wales=100.

Caribbeans showed a significant deficit (standardised mortality ratio 33). These patterns were also apparent for anencephaly. Differences between groups were much smaller for spina bifida, with the lowest ratios in the Caribbean and West African groups. Mortality from other central nervous system anomalies was high in all groups of Asian origin, being highest in Pakistanis (standardised mortality ratio 379).

Infants of mothers born in Pakistan showed the highest mortality for most other anomalies examined (table 5), particularly for genitourinary (standardised mortality ratio 268) and limb and musculoskeletal anomalies (standardised mortality ratio 362). Other groups of Asian origin also showed high mortality from anomalies of the circulatory, digestive, and genitourinary systems. Chromosomal anomalies showed relatively smaller intergroup variations.

Discussion

This study is based on the underlying cause of death and does not include other abnormalities mentioned on death certificates. As a study of mortality, it also does not include defects compatible with life beyond the first year. The reliability of mortality as a measure of birth prevalence will therefore depend on the type of anomaly under consideration. Furthermore, rates of survival for similarly affected infants may vary among the different ethnic groups, thereby affecting the proportions dying within the first year.

Our findings showed significantly higher mortality from malformations in infants of Asian origin compared with infants of mothers born in this country. The overall rate in Indians and Pakistanis was 25% and 121% higher respectively than the rate among mothers in social class V born in the United Kingdom. Compared with mothers born in the United Kingdom in social class I, the rates among Indians and Pakistanis were higher by 68% and 196% respectively. Of particular concern is the incidence of malformation among Pakistani infants. Differences in mortality between Pakistanis and other groups of Asian origin were considerably larger than those between the latter and the indigenous population.

Most East Africans are of Indian origin, which may explain why their standardised mortality ratios correspond to those for Indians. The low standardised mortality ratios for West Africans, on the other hand, showed greater agreement with those for Caribbeans. West Africans and Caribbeans were different from the other groups studied in that they did not show rising mortality from social class I to V, a finding noted also in a study of perinatal mortality among mothers delivered at St Thomas's Hospital, London, during 1969–76.[19]

These results are broadly consistent with the patterns reported for 1976–80,[4] even though the earlier analysis did not adjust for social class. In both periods Pakistanis had high standardised mortality ratios for most types of defects, specially for central nervous system and musculoskeletal anomalies. In both periods Caribbeans had low standardised mortality ratios, particularly for central nervous system defects and most notably for spina bifida. As in 1976–80, spina bifida showed less variation among groups than other malformations. In this study chromosomal anomalies, not included in the earlier analysis, showed the least variation.

Our findings are generally consistent with the results of other studies. A low incidence of serious malformations has been reported for populations of Afro-Caribbean origin in America, Africa, Britain, and elsewhere, even after migration.[1,2,5–10] In particular, the significantly low mortality from central nervous system anomalies among Caribbeans in this study is consistent with other reports of a low rate of neural tube defects in negroid subjects. On the other hand they are consistently found to have higher rates for some less severe abnormalities, in particular for polydactyly and supernumerary nipples,[6–9,11] conditions which could not be examined in a study of mortality such as this.

For the Asian groups, data from the countries of origin is sparse except in the case of India. Most of the Indian data comes from local hospital based studies, and the indices of measurement are not uniform. Comparisons with our results therefore were not readily possible. Previous studies in England have reported a high incidence of malformations in Asian infants generally[1,2,10,12] and among Pakistanis in particular.[3]

Discussion about the causes underlying ethnic differences in malformations has on the whole been inconclusive, because the aetiology of most congenital anomalies is both complex and unknown. Many factors in addition to genetic susceptibility are associated with incidence: season of birth, social class, maternal age, parity, sex of child, and nutrition. Antenatal screening and social and cultural practices (for instance, smoking, alcohol consumption, diet, and consanguinity) also influence incidence. The relative impact of environmental factors and genetic susceptibility on incidence is said to vary both with the type of anomaly and with the populations under consideration.[5]

The low overall incidence of defects (neural tube defects in particular) in negroid subjects is generally attributed to genetic factors and mixed descent.[5,6,8,13] It has also been suggested that different rates of fetal maturation and skeletal growth during critical periods of fetal development could explain some of the differences observed between white and negroid subjects.[8]

Reasons for variations within the Asian community have scarcely been investigated. Of particular interest are the differences observed here between Pakistanis and Indians. Consanguinity, high fertility, and childbearing late into the reproductive period, are more common in Pakistanis than Indians,[2,3,10,20] and could be contributory factors. Neural tube defects are thought to be more common in women of high fertility.[5]

It was not possible to estimate the impact of antenatal screening on these ethnic differences as abortion statistics are not available by country of birth. Induced abortions for fetal abnormalities contributed to the decline in central nervous system anomalies (anencephaly in particular) that has been evident in England and Wales since the early 1970s.[21–23] The number of terminations of pregnancy was, however, insufficient to account for more than a small part of the national decline.[23] The notification rate for Down's syndrome has shown little change since 1970, except among older mothers.[22] The notification rate for all malformations, though concealing variations between individual conditions, has remained steady during the years 1976–85.[24] The contribution of antenatal screening to ethnic differences in malformations is likely to be small, therefore, except in the case of central nervous system anomalies.

These differentials between similar ethnic groups offer an opportunity to gain new insights into the aetiology of congenital malformations. The costs, both human and economic, of such high malformation rates will increase as the population at risk grows. The Pakistani population in Great Britain, estimated by the Labour Force Survey to be 380 000 during 1983–5, is young and has high fertility, with about 14 000 births a year. The number of infants at risk is therefore large, and likely to increase. There are an additional 23 000 births a year to other mothers of Asian origin (including East Africans). Our results highlight the need for further research among these high risk groups to investigate and identify some of the underlying causes which contribute to these differences.

References

[1] Leck I. Ethnic differences in the incidence of malformations following migration. *British Journal of Preventive and Social Medicine* 1969;**23:**166–73.

[2] Terry PB, Bissenden JG, Condie RG, Mathew PM. Ethnic differences in congenital malformations. *Arch Dis Child* 1985; **60:**866–8.

[3] Gillies DRN, Lealman GT, Lumb KM, Congdon P. Analysis of ethnic influence on stillbirths and infant mortality in Bradford 1975–81. *J Epidemiol Community Health* 1984;**38:**214–7.

[4] Balarajan R, McDowall M. Mortality from congenital malformations by mother's country of birth. *J Epidemiol Community Health* 1985;**39:**102–6.

[5] Leck I. The geographical distribution of neural tube defects and oral clefts. *Br Med Bull* 1984;**40:**390–5.

[6] Erickson JD. Racial variations in the incidence of congenital malformations. *Ann Hum Genet* 1976;**39:**315–20.

[7] Altemus LA, Ferguson DA. Comparative incidence of birth defects in negro and white children. *Pediatrics* 1965;**36:**56–61.

[8] Polednak AP. Birth defects in blacks and whites in relation to prenatal development: a review and hypothesis. *Hum Biol* 1986;**58:**317–35.

[9] Chung CS, Myrianthopoulos NC. Racial and prenatal factors in major congenital malformations. *Am J Hum Genet* 1968;**20:** 44–60.

[10] Terry PB, Condie RG, Settatree RS. Analysis of ethnic differences in perinatal statistics. *Br Med J* 1980;**281:**1307–8.

[11] Christianson RE, Van Den Berg BJ, Milkovich L, Oechsli FW. Incidence of congenital anomalies among white and black live births with long-term follow-up. *Am J Public Health* 1981;**71:** 1333–9.

[12] Clarke M, Clayton DG. Quality of obstetric care provided for Asian immigrants in Leicestershire. *Br Med J* 1983;**286:**621–3.

[13] Neel JV. A study of major congenital defects in Japanese infants. *Am J Hum Genet* 1958;**10:**398.

[14] Office of Population Censuses and Surveys. *Mortality statistics, perinatal and infant: social and biological factors, 1981.* Series DH3 No 13. London: HMSO, 1985.

[15] Office of Population Censuses and Surveys. *Immigrant mortality in England and Wales 1970–78: causes of death by country of birth.* Studies on Medical and Population Subjects No 47. London: HMSO, 1984.

[16] Office of Population Censuses and Surveys. *Labour force survey 1981: country of birth and ethnic origin.* OPCS Monitor, ref LFS 83/1 and PP1 83/1. London: HMSO, 22 February 1983.

[17] Office of Population Censuses and Surveys. *Labour force survey 1984: country of birth, ethnic group, year of entry, and nationality.* OPCS Monitor, ref LFS 85/1 and PP1 85/3. London: HMSO, 17 December 1985.

[18] Office of Population Censuses and Surveys. *Estimating the size of the ethnic minority populations in the 1980s.* Population Trends 44. London: HMSO, Summer 1986: 23–7.

[19] Robinson MJ, Palmer SR, Avery A, James CE, Beynon JL, Taylor RW. Ethnic differences in perinatal mortality—a challenge. *J Epidemiol Community Health* 1982;**36:**22–6.

[20] Office of Population Censuses and Surveys. *Births by birthplace of parent 1985.* OPCS Monitor, ref FM1 86/5. London: HMSO, 29 July 1986.

[21] Bradshaw J, Weale J, Weatherall J. *Congenital malformations of the central nervous system.* Population Trends 19. London: HMSO, Spring 1980: 13–18.

[22] Weatherall JAC. A review of some effects of recent medical practices in reducing the numbers of children born with congenital abnormalities. *Health Trends* 1982;**14:**85–8.

[23] Leck I. Spina bifida and anencephaly: fewer patients, more problems. *Br Med J* 1983;**286:**1679–80.

[24] Office of Population Censuses and Surveys. *Congenital malformations, 1985.* OPCS Monitor ref MB3 86/2. London: HMSO, 7 October 1986.

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Safety trial of heated factor VIII concentrate (8Y)

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SUMMARY Seventeen previously untreated boys with haemophilia A were treated with high purity heat treated factor VIII concentrate (8Y) for up to 36 months. Liver function tests were assessed monthly. No boy's serum has been shown to contain HIV antibodies and no increases in alanine transaminase activity have been detected. In only one patient was a single rise in aspartate transaminase activity noted, and this was without a corresponding rise in alanine transaminase. A second patient's serum contained hepatitis B core antibody transiently. It was thought likely in both cases that the abnormalities reflected intercurrent infections rather than disease associated with transfusion.

The physical treatments used in the production of 8Y seem to inactivate the agent(s) responsible for non-A, non-B hepatitis and HIV transmission by transfusion of factor VIII has been abolished. There are, however, problems associated with conducting safety trials in young haemophiliac patients.

Although the introduction of factor concentrates has revolutionised the treatment of bleeding episodes in haemophilia, transmission of viruses remains a serious concern. Until recently non-A, non-B hepatitis had nearly a 100% incidence in patients previously treated with large pool unheated concentrates, whether they were drawn from paid or volunteer donors, implying inevitable contamination of such products.[1 2] The introduction of dry heat treatment of freeze dried factor VIII concentrate to inactivate HIV, has shown little or no effect on reducing the risk of transmission of non-A, non-B hepatitis to those at greatest risk, namely previously untreated haemophiliacs.[3 4] Heating in solution—wet heat treatment of factor VIII concentrate—may reduce the risk of non-A, non-B hepatitis to about 30%,[5] or may even abolish it.[6] Other methods of viral inactivation may also reduce the incidence of non-A, non-B hepatitis including solvent/detergent treatment,[7] or heating in hot vapour,[8] though the latter process failed to inactivate hepatitis B completely.[8]

In 1985 the Blood Products Laboratory (Elstree) introduced a new factor VIII concentrate, type 8Y, which is heated at 80°C for 72 hours. Dry heat treatment at 60°C had little effect on the transmission of non-A, non-B hepatitis,[3] and 80°C is a higher temperature than previously reported in attempts to inactivate non-A, non-B hepatitis[9] and 'model' retroviruses.[10] Initial results using this product were encouraging, and there were no cases of non-A, non-B hepatitis reported.[11]

We have therefore studied 17 previously untreated haemophiliac boys for up to 36 months after infusion of factor VIII concentrate 8Y to assess the risks and incidence of viral transmission. We will also address the problems of performing safety studies in young haemophiliac boys.

Patients and methods

Seventeen boys were admitted to the study from a single paediatric haemophilia centre during the period July 1985 to April 1988. Patient selection complied with the criteria recommended by the International Committee on Thrombosis and Haemostasis (Miami, Florida 1984) for evaluating treated clotting factor concentrates. Accordingly no boy had been previously exposed to any blood nor blood products, had normal transaminase activities, and no markers of hepatitis B infection or clinical evidence of liver disease at the beginning of the study. All were negative for HIV antibody. All the boys had haemophilia A; 13 had severe haemophilia (VIII:C<0·01 U/ml) and four had mild haemophilia (VIII:C 0·08–0·14 U/ml). Their ages ranged from 2 weeks to 16 weeks, and the mean age of the severely affected group at entry to the study was 14 months. All the boys were immunised against hepatitis B infection at or around the time of the first infusion with 8Y. Treatment was given according to the clinical judgment of the attending physician. Informed parental consent was gained in all cases.

CONCENTRATE

Dried factor VIII fraction, high purity, heat treated, type 8Y, became available in 1985. It is prepared from large pool fresh frozen plasma from unpaid donors of the National Blood Transfusion Service in England and Wales. Each unit is screened to make sure that it does not contain hepatitis B surface antigen (HBsAg) by third generation tests, but it is not screened for surrogate markers of non-A, non-B hepatitis (serum aminotransferase activities) or hepatitis B core antibody (HBcAb). Since 1986 plasma has also been screened to make sure that it does not contain HIV antibodies. After lyophilisation, the concentrate is heated in a dry state at 80°C for 72 hours in its final container. It is of high purity and high specific activity, in excess of 2 IU factor VIII/mg protein. Studies in our unit have indicated that the biological half life and recovery of VIII:C and its effectiveness in stopping bleeding episodes in patients with haemophilia are similar to those of unheated concentrates (unpublished observations). Twenty production batches of 8Y have been used in the study over a 36 month period during 1985–8.

DIAGNOSTIC AND FOLLOW UP CRITERIA

Blood samples were collected before the first infusion of 8Y, and then at monthly intervals irrespective of whether additional infusions of concentrate were given. This protocol is in contrast to the International Committee on Thrombosis and Haemostasis (Miami, Florida 1984) recommendations that testing should be done every two weeks for the first four months after the initial infusion. Monthly testing was adopted, as most parents would only consent to blood samples every four weeks unless treatment was required before the routine monthly sampling. Full compliance with this monthly regimen was sought. Samples were tested at each occasion for alanine transaminase, and alkaline phosphatase activities, bilirubin concentration, antibody to HIV hepatitis B markers, and a full blood count was also done.

Non-A, non-B hepatitis was defined as alanine transaminase activity more than 2·5 times the upper limit of normal for age on at least two separate occasions during the follow up period, other causes of hepatitis having been excluded.

Results

Demographic data, baseline VIII:C concentrations, total dose of concentrate, number of batches, initial indications for infusion, and duration of follow up are shown in the table. All the boys have remained negative for HIV antibody.

Transaminase activities were measured on 348 separate occasions. No increases in alanine transaminase activity were detected but one child had an isolated increase in aspartate transaminase activity. This rose from 27 IU/l to 131 IU/l six weeks after treatment with a new batch of 8Y. The abnormal aspartate transaminase activity returned to normal within 12 days. Viral serology for hepatitis A, Epstein-Barr virus, and cytomegalovirus was

Table *Clinical details of the patients studied*

Case No	*VIII:C (U/ml)*	*Age at entry*	*Indication for treatment*	*Total dose of 8Y (units)*	*No of treatments*	*No of batches*	*Period studied (months)*
1	<0·01	13 Months	Injury to finger	66,050	127	13	36
2	<0·01	13 Months	Hepatitis B vaccination	33,255	93	17	31
3	<0·01	17 Months	Muscle bleed	62,525	127	14	30
4	<0·01	11 Months	Head injury	68,125	151	17	28
5	0·14	15 Years	Fractured femur	74,635	34	4	25
6	<0·01	9 Months	Haemarthrosis	31,850	64	9	25
7	<0·01	3 Months	Hand injury	27,205	97	10	21
8	<0·01	39 Months	Muscle bleed	28,515	40	9	20
9	0·09	9 Years	After operation	15,635	20	2	19
10	<0·01	15 Months	Head injury	24,610	43	7	18
11	<0·01	15 Months	Haemarthrosis	31,840	67	5	17
12	<0·01	5 Months	Ear injury	14,540	38	7	17
13	<0·01	21 Months	Haemarthrosis	10,440	24	6	17
14	0·10	6 Years	Head injury	1,920	2	1	16
15	<0·01	17 Months	Mouth bleed	27,550	69	7	15
16	<0·01	2 Weeks	Umbilical bleed	1,370	5	4	9
17	0·14	5 Years	After operation	10,870	12	2	4

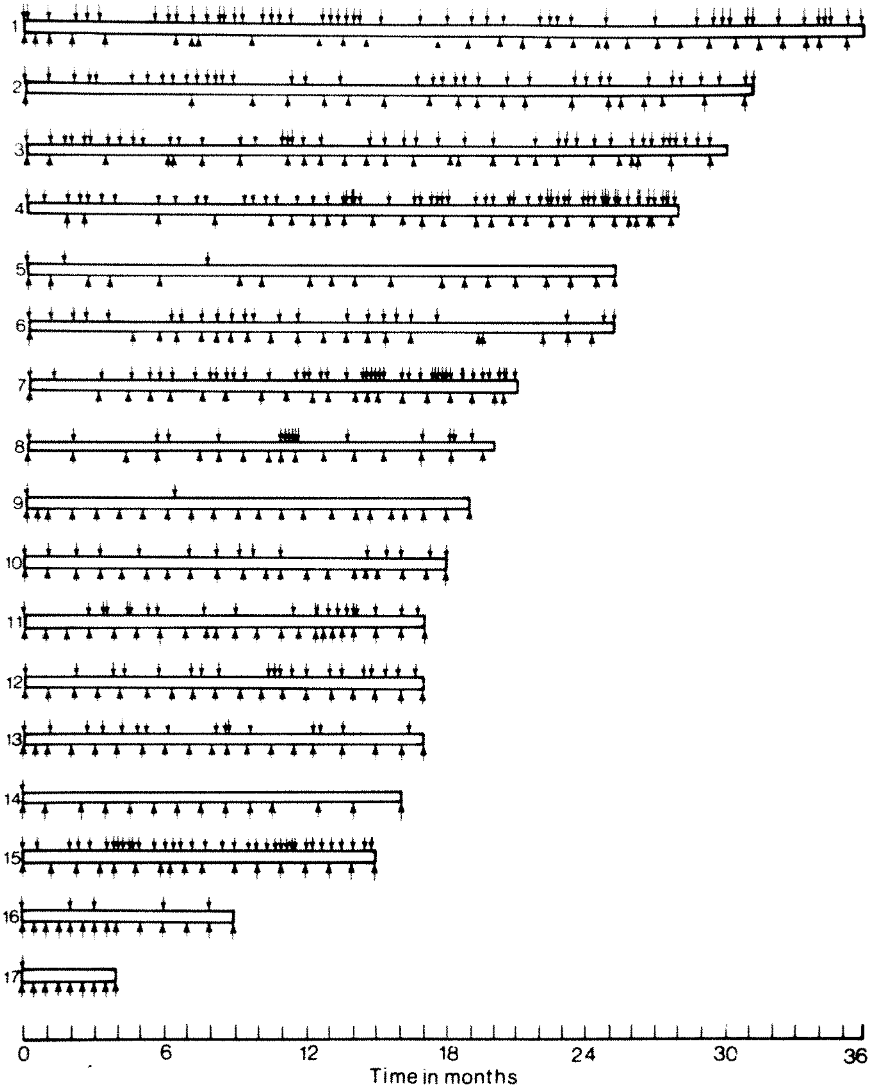

Fig 1 *Timing of testing in relation to treatment episodes. Each bar represents the period of follow up of each boy. Arrows above the bar represent treatment episodes. Arrows below the bar represent points at which blood tests were carried out.*

negative on both acute and convalescent sera. The child remained well throughout with no signs of liver disease or any other illness.

All the boys were immune to hepatitis B after immunisation. In one of the 17 a transient HBcAb was observed; the temporal pattern and equivocal or low concentration of this antibody suggested that it may have been passively acquired. He has had no clinical or biochemical evidence of liver disease, and all other family members are non-immune to hepatitis B. He remains HBsAb positive and HBcAb negative.

Fig 1 shows the temporal relation of testing to treatment episodes. In some of the patients entered into the study early on it proved impossible to adhere strictly to a monthly regimen, particularly in the younger children. The mean monthly attendance for the total period of study was 85%, and 94% for

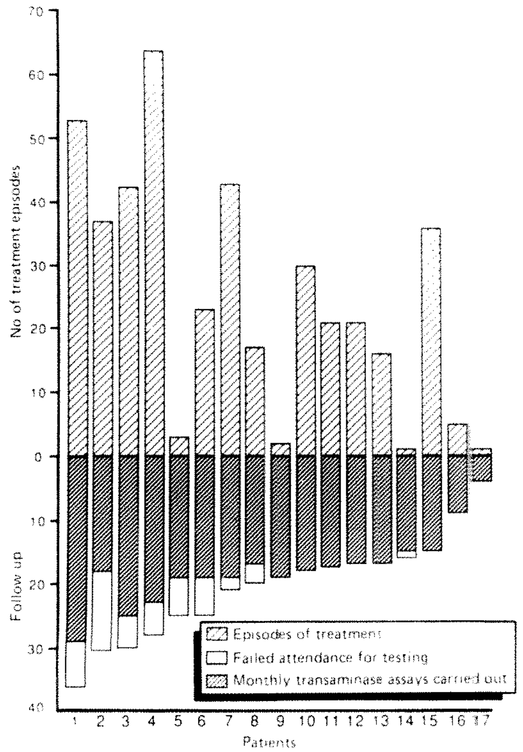

Fig 2 *Number of treatment episodes shown in relation to number of montly transaminase assays carried out and failed monthly attendances for testing. Total length of the downward bar is equivalent to the total period of follow up (months).*

the last year of the study. Failure to attend was usually because the boys were well, and the parents were consequently reluctant to bring them to the hospital for blood testing (fig 2).

Discussion

As experiments in chimpanzees do not provide a safe model for the infectivity of hepatitis in humans[3 12] and non-A, non-B hepatitis is commonly asymptomatic in its acute stages, the only way to show infectivity has been to carry out clinical trials. Patients most at risk of developing non-A, non-B hepatitis are those who have never previously been exposed to any blood or blood products. Such patients are usually young boys. Carrying out clinical trials in young children, particularly when frequent venepuncture is required, poses ethical problems as also noted by other investigators.[5]

Strict adherence to two weekly testing regimens (as recommended by the International Committee on Thrombosis and Haemostasis) in small children requires considerable parental cooperation and altruism. A particular problem in this type of study is the lack of any immediate benefit to the patient from the regular blood testing. Parents are often unwilling to submit their children to frequent and sometimes difficult and traumatic venepuncture, especially if the child is well at the time. Collection of capillary blood offers an alternative and less invasive method of blood sampling. Capillary blood sampling is, however, potentially more hazardous to other personnel because of blood spillage and contamination. Because of this staff may be unwilling to collect capillary blood samples from these patients until a product's safety has been established. Venepuncture is therefore required, and even carried out by experienced paediatric staff may be difficult in young boys. Parental anxiety that veins may be damaged and that treatment of acute bleeds may not therefore be possible must be allayed. As liver disease is still a serious problem in haemophilia, obtaining information about the safety of products is of prognostic importance for their sons. Detailed information, encouragement of parents to take an active part in treatment, and regular encouragement by telephone or letter of defaulters to attend, have all improved compliance and achieved fairly strict monthly testing in most of the young boys.

It has been argued that monthly blood testing, until four months after the start of treatment will identify most patients who develop non-A, non-B hepatitis,[11] and so it is now becoming more widely acknowledged that in young boys strict monthly testing, if rigidly achieved, is acceptable to assess risk (Proceedings World Federation of Haemophilia, 1988).

We have found that in previously untreated boys receiving 8Y, alanine transaminase activities have remained normal throughout the three year study period; this suggests that 8Y does not transmit non-A, non-B hepatitis in such young children and that the heating process (80°C for 72 hours) seems to inactivate the agent or agents responsible for transmission of non-A, non-B hepatitis. In the United Kingdom the incidence of non-A, non-B hepatitis in the donor population was previously estimated as 0·3%.[13] It is likely that all production batches of 8Y will carry at least one donation capable of transmitting non-A, non-B hepatitis, even when diluted with thousands of non-infective donations. We presented further evidence for the efficacy of this heat treatment process when we showed that a reduced incidence of parvovirus B19 infection, a heat resistant virus, was observed in the boys treated with 8Y compared with a previous group of boys treated with unheated large pool concentrates (MD Williams, AC Bedall, K Al Rubei, *et al.* Transmission of parvovirus B19 by clotting factor concentrate. Abstract presented at 20th Congress of International Society of Blood Transfusion, London, 1988). There was also no apparent transmission of HIV as all boys remained negative for HIV antibody, thus providing circumstantial evidence that HIV transmission has been abolished. Despite the heat sensitivity of HIV being well established,[10] however, we cannot be sure that any batch was actually infected with HIV because the incidence of HIV seropositivity in the donor population is only 0·002% and the risks of HIV transmission are further reduced by the introduction of a national HIV antibody screening programme in the United Kingdom.

'Wet' heated products, whether heated in hot vapour or moistened, have been proposed as being safer than products heated when dry,[5 6 8] usually at 60°C, and that such products are to be preferred because 'dry' heating is ineffective in preventing hepatitis. Our data support the contention that 'dry' heating of factor VIII concentrate made from plasma from British volunteers (HIV antibody and hepatitis B antigen screened and negative) at 80°C for 72 hours is a safe and effective process for viral inactivation. In the future, however, safety studies in previously untreated patients will inevitably have to be undertaken in young newly diagnosed haemophiliacs. As haemophilia is a rare disorder such studies will inevitably be small, and consequently the statistical confidence limits of such studies will be limited. Further study on more untreated patients, adhering as closely as possible to International Committee on Thrombosis and Haemostasis recommendations, is required to improve statistical confirmation of product safety. More frequent assessment of alanine transaminase activity is now feasible using capillary blood samples, because the safety of 8Y shown by this study has allayed the anxieties of staff about performing capillary blood sampling.

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References

[1] Fletcher ML, Trowell JM, Craske J, Pavier K, Rizza CR. Non-A, non-B hepatitis after transfusion of factor VIII in infrequently treated patients. *Br Med J* 1983;**287**:1754–7.

[2] Kernoff PBA, Lee CA, Karayiannis P, Thomas HC. High risk of non-A, non-B hepatitis after first exposure to volunteer or commercial clotting factor concentrates: effects of prophylatic immune serum globulin. *Br J Haematol* 1985;**60**:469–79.

[3] Colombo M, Carnelli V, Gazengel C, Mannucci PM, Savidge GF, Schimpf K. Transmission of non-A, non-B hepatitis by heat treated factor VIII concentrate. *Lancet* 1985;**ii**:1–4.

[4] Preston FE, Hay CRM, Dewar MS, Greaves M, Triger DR. Non-A, non-B hepatitis and heat treated factor VIII concentrates. *Lancet* 1985;**ii**:213.

[5] Kernoff PBA, Miller EJ, Savidge GF, Machin SJ, Dewar MS, Preston FE. Reduced risk of non-A, non-B hepatitis after a first exposure to 'wet heated' factor VIII concentrate. *Br J Haematol* 1987;**67**:207–11.

[6] Schimpf K, Mannucci PM, Kreutz W, *et al.* Absence of hepatitis after treatment with pasteurized factor VIII concentrate in patients with haemophilia and no previous transfusions. *N Engl J Med* 1987;**316**:918–22.

[7] Horowitz MS, Rooks C, Horowitz B, Hilgartner MW. Virus safety of solvent/detergent-treated antihaemophilic factor concentrate. *Lancet* 1988;**ii**:186–8.

[8] Mannucci PM, Zanetti AR, Colombo M. Study Group of the Fondazione Dell 'Emofilia. Prospective study of hepatitis after factor VIII concentrate exposed to hot vapour. *Br J Haematol* 1987;**68**:427–30.

[9] Dolana G, Hollinger FB, Thomas W, Gyorkey F. Continued observation on the effect of a heating procedure on the inactivation of non-A, non-B hepatitis and hepatitis B viruses in clotting factor concentrate. *Thromb Haemost* 1983;**50**:115.

[10] Levy JA, Mitra GA, Wong ME, Mozen MM. Inactivation by wet and dry heat of AIDS-associated retroviruses during factor VIII purification from plasma. *Lancet* 1985;**i**:1456–7.

[11] Study Group of UK Haemophilia Centre Directors on Surveillance of Virus Transmission By Concentrates. Effect of dry-heating of coagulation factor concentrates at 80°C for 72 hours on transmission of non-A, non-B hepatitis. *Lancet* 1988;**ii**: 814–6.

[12] Hollinger FB, Dolana G, Thomas W, Gyorkey F. Reduction in risk of hepatitis transmission by heat treatment of human factor VIII concentrate. *J Infect Dis* 1984;**150**:250–62.

[13] Collins JD, Bassendine MF, Codd AA, Collins A, Ferner RE, James OFW. Prospective study of post-transfusion hepatitis after cardiac surgery in a British centre. *Br Med J* 1983;**287**: 1422–4.

[14] Gunson HH, Rawlinson VI. HIV antibody screening of blood donations in the United Kingdom. *Vox Sang* 1988;**54**:34–8.

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Screening for anaemia: evaluation of a haemoglobinometer

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SUMMARY The HemoCue haemoglobinometer was evaluated as part of a programme to screen children aged 11–36 months for anaemia when they attended a routine immunisation clinic. Paired capillary blood samples were collected and measured on the haemoglobinometer and on a Coulter counter model S. Measurements made from bottle collected capillary samples were more precise than those made from single drops of blood. After compensation for a fixed bias the haemoglobinometer had a sensitivity of 0·85 and a specificity of 0·94 when used to detect anaemia.

Careful attention must be paid to the method of blood collection. We suggest that cuvettes are filled from bottle collected capillary samples rather than single blood droplets taken direct from the finger. The haemoglobinometer was reliable, simple to use with the minimum of training, and proved to be suitable for use in a community child health clinic.

Iron deficiency anaemia in children retards the attainment of developmental milestones, causes growth to slow down, and increases the risk of infection.[1 2] These factors have led to calls for routine screening for anaemia in infants.[3–5] The ability to measure haemoglobin concentration quickly, accurately, and with minimal trauma will form an important part of any screening programme.

The HemoCue haemoglobinometer (Clandon Scientific) is a portable machine that measures haemoglobin by photometric assay with disposable plastic cuvettes. Only 10 µl of blood is required and the result is shown on a liquid crystal display within 60 seconds. It is accurate and precise when used on mixed venous blood in laboratory conditions.[6]

This study was designed to evaluate the system in an infant welfare clinic using finger prick blood samples.

Subjects and methods

Parents of children aged 11 to 36 months attending four different immunisation clinics in east London were invited to participate in a programme to screen their child for anaemia. Parents were approached on arrival at the clinic and were given a short verbal and written description of the test. The verbal information was always given by the same person (AFM). Translators were present, and the written description was available in English, Urdu, Bengali, Punjabi, and Gujarati. The screening test was always undertaken after immunisation.

Finger prick blood was sampled using a spring loaded lancet device (Autolet). No preparation (such as warming the finger) was necessary. This blood was collected into a 1·8 ml bottle containing edetic acid. A cuvette was filled directly from the finger (direct method) and a second cuvette was filled from the specimen in edetic acid after it had been carefully agitated (bottle method). Cuvettes were measured immediately they had been filled. The remainder of the specimen in edetic acid was sent for routine laboratory analysis on a Coulter counter 'S' at the Whipps Cross Hospital haematology laboratory.

Results that were below the 95% reference range for haemoglobin concentration (<110 g/l) were classified as anaemic.[7]

Standardisation of the HemoCue to the Coulter counter was checked during the study using four laboratory standards (range 83–114 g/l). Two cuvettes were measured from each standard. This indicated a mean (SEM) positive bias of 1·4 (0·9) g/l. The 95% confidence interval was 5·1 g/l above and below the mean. Calibration was checked daily using a control cuvette and was never found to vary by more than

1 g/l above or below the mean. No recalibration was undertaken during the course of the study.

Accuracy was measured for each method by calculating the mean and standard deviation of differences between results from bottle method and Coulter counter or direct method and Coulter counter.[8] The mean was a measure of bias, and the 95% precision range was the mean plus or minus 1·96 multiplied by the standard deviation.

If children were found to be anaemic they were referred for further assessment and follow up.

Approval was obtained from both hospital and district ethics committees.

Results

Of those offered the test, 65% agreed to be investigated.

A total of 102 samples measured by both direct and bottle methods and Coulter counter were collected. Figs 1 and 2 show the accuracy and precision of each method. Both methods show a positive bias of between 3 and 4 g/l, but the bottle method was more precise.

There was no significant difference in the precision of either method in the first half of the study compared with the second half. Experience of the operator was therefore not a factor.

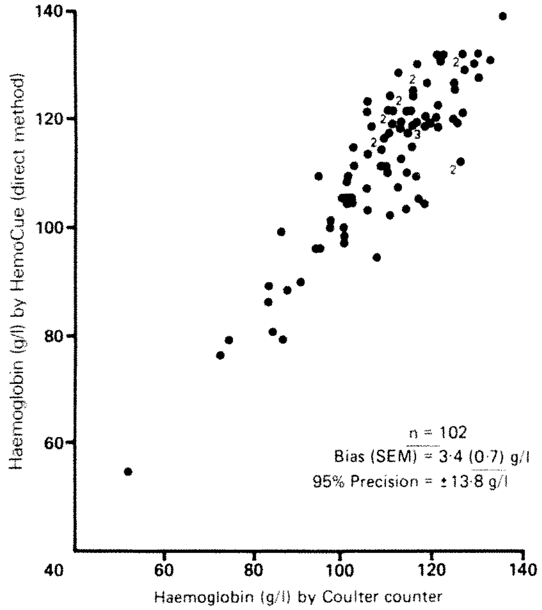

Fig 1 *Haemoglobin concentration measured by HemoCue after direct collection and by Coulter counter 'S'.*

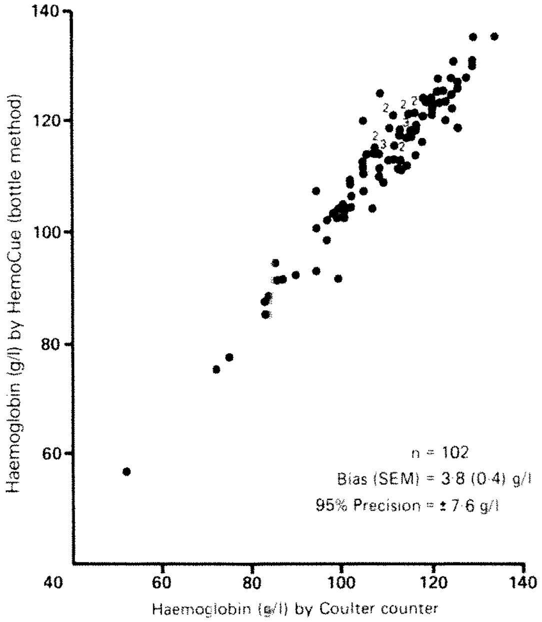

Fig 2 *Haemoglobin concentration measured by HemoCue after bottle method collection and by Coulter counter 'S'.*

Table 1 *Validity of each method before and after correction for bias*

Method of collection of blood	*Validity*	
	Sensitivity (proportion of cases correctly classified anaemic)	*Specificity (proportion of cases correctly classified healthy)*
Direct	70	90
Direct, corrected	80	82
Bottle	75	100
Bottle, corrected	85	94

Correction for bias = −4 g/l from each HemoCue result.

Table 1 shows the sensitivity and specificity of the methods in both healthy and anaemic patients. The effect of compensation for bias by subtracting 4 g/l from each HemoCue result is shown. Table 2 shows the detailed results using the bottle method compensated for bias.

Haemostasis was achieved easily by pressing on a clean paper tissue. Adhesive plasters were not used because of concern that young children might swallow them.

There were no complications after the finger prick. The investigation was well received by parents. The haemoglobinometer was simple to use, easily

Table 2 *Results using bottle method, corrected for bias*

HemoCue results	*Coulter results*		*Total*
	Anaemic (haemoglobin <110 g/l)	*Healthy (haemoglobin ≥110 g/l)*	
Anaemic (haemoglobin <110 g/l)	34	4	38
Healthy (haemoglobin ≥110 g/l)	6	58	64
Total	40	62	102

portable, and reliable. No machine failure occurred during the study.

Discussion

A high percentage of the population served by Waltham Forest health authority originate from India and Pakistan. There is much social deprivation. A previous study from this health authority found a prevalence of iron deficiency anaemia of 25% in 1 year old Asian children.[9] A campaign to find and treat iron deficiency anaemia was therefore considered a priority for the community paediatric service.

Any screening test should be accurate (with a low number of false positives and false negatives), acceptable, and inexpensive. Unfortunately, no single investigation can reliably separate all those with and without iron deficiency. Haemoglobin concentration is the most conveniently measured variable, although the high incidence of haemoglobinopathies in some groups complicates this. A haemoglobin concentration of less than 110 g/l was regarded as an indication for further assessment.[7]

The use of single finger prick samples (direct method) was found to be unacceptably imprecise. The large variation in finger prick sample values compared with results from the Coulter counter was probably caused by rouleaux formation and haemodilution at the finger tip. The measurement of capillary blood collected in a bottle seemed to compensate for these errors and considerably improved accuracy without adding to technical difficulty. This technique makes the rapid and accurate measurement of haemoglobin possible in a busy clinic.

An important error was a fixed positive bias which significantly reduced accuracy. The HemoCue operating manual recommends the use of a single standard specimen to check the calibration, in addition to daily use of the control cuvette provided. In this study four standard samples were used. This calibration did not uncover the bias that was subsequently noted over 102 pairs of specimens. A spontaneous change in the performance of the haemoglobinometer is unlikely to explain this observation, as at no time did the control cuvette indicate a change in calibration. The reason this bias was undetected was probably the small number of standardisation measurements used, as both the HemoCue and Coulter counter have operating errors. To allow for this we recommend that initial calibration should be made using at least 30 measurements from a number of standard specimens with a wide range of values.[8] The mean difference between HemoCue results and sample values will indicate the machine bias, which can then be recalibrated accordingly. The control cuvette is then measured on the machine and its value redesignated. Subsequent quality control needs to be monitored regularly.

After adjustment for bias, the bottle method correctly classified 85% of the anaemic and 94% of the healthy cases. The use of the bottle method makes further investigation easier by collecting a specimen that can be sent for analysis if the haemoglobin concentration is low on first screening.

Taking finger prick samples with a spring loaded lancet was simple and caused minimal upset. For a routine screening test, capillary blood sampling has considerable advantages over venepuncture. The speed and ease of a finger prick sample is likely to ensure satisfactory uptake. Venepuncture in infants is often technically difficult, more time consuming, and usually requires two members of staff. A technique that only requires a single operator clearly has advantages in any service constrained by lack of funds or personnel.

It is difficult to estimate the cost of a screening programme such as this. The haemoglobinometer cost £434, and each microcuvette costs 25p and can be used only once. Additional costs will be that of bottles containing edetic acid and the lancets. Greater expense will be for doctors' time, and the expense of follow up investigations and counselling. The fact that only one person is needed to administer the test makes it attractive, and the immediate availability of results means that counselling and possibly treatment can be started at the same interview. This saved considerable administrative time and was popular with parents.

It has been stressed that equipment intended for primary health care should be evaluated under normal working conditions.[6] This study evaluated the HemoCue in the sort of clinic in which it will be used. It is robust, portable, and simple to use. It operates on rechargeable batteries and therefore does not need a power socket. Careful attention must be paid to methods of collecting blood and calibrating the machine for optimal accuracy.

We conclude that the collection of blood into a bottle for a mixed sample, rather than direct filling from the finger prick, is more accurate and reliable. The HemoCue is a useful instrument for the routine screening of children for iron deficiency anaemia within the community paediatric service although those found to be anaemic require clinical assessment, precise laboratory diagnosis, and careful follow up.

We thank Dr C D'Silva and the medical laboratory scientific officers from Whipps Cross Hospital haematology department for analysing Coulter specimens and for assistance with calibration, Mr J Clegg of Clandon Scientific, Mrs Inda Ford for translation, and Dr P Thompson for advice and encouragement.

References

[1] Aukett MA, Parks YA, Scott PH, Wharton BA. Treatment with iron increases weight gain and psychomotor development. *Arch Dis Child* 1986;**61**:849–57.

[2] Lozoff B, Brittenham GM, Wolf AW, *et al*. Iron deficiency anaemia and iron therapy: effects on infant developmental test performance. *Pediatrics* 1987;**79**:981–95.

[3] Illingworth RS. Anaemia and child health surveillance. *Arch Dis Child* 1986;**61**:1151–2.

[4] Addy DP. Happiness is: iron. *Br Med J* 1986;**292**:969–70.

[5] Editorial. Iron deficiency—time for a community campaign? *Lancet* 1987;**i**:141–2.

[6] Neville RG. Evaluation of portable haemoglobinometer in general practice. *Br Med J* 1987;**294**:1263–5.

[7] Dallman PR, Reeves JD. Laboratory diagnosis of iron deficiency. In: Stekel A, ed. *Iron nutrition in infancy and childhood*. Nestlé Nutrition Workshop Series, Vol 4. New York: Raven Press, 1984.

[8] Altman DG. Statistics and ethics in medical research. In: Gore SM, Altman DG, eds. *Statistics in practice*. London: British Medical Association, 1982:1–24.

[9] McEnery G, Rao KPS. The effectiveness of antenatal education of Pakistani and Indian women living in this country. *Child Care Health Dev* 1986;**12**:385–99.

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Egg and breast milk based nitrogen sources compared

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SUMMARY A nitrogen source based on egg protein (Vamin 9 glucose) and an alternative with an amino acid profile more similar to breast milk (Vaminolact), were compared in 14 parenterally fed infants. Subjects were randomly allocated to receive one or other amino acid solution, but were otherwise given identical diets. At the start of the study the two groups did not differ significantly in postconceptual age, postnatal age, or weight. Over a six day study period on a stable intake of intravenous nutrients there was no significant difference in growth or nitrogen retention between the two groups. Plasma amino acid profiles in those receiving Vamin 9 glucose, however, were frequently abnormal. Notably, mean concentrations of potentially neurotoxic phenylalanine and tyrosine were significantly higher (140% and 420%, respectively) in patients fed Vamin 9 compared with those given Vaminolact. An amino acid solution based on the composition of breast milk protein therefore brings plasma amino acid profiles during parenteral nutrition closer to those found in breast fed infants, and reduces in particular, the risks of hyperphenylalaninaemia and hypertyrosinaemia.

Over the past 20 years intravenous feeding has become a standard form of nutritional support for sick infants. Although the indications for its use in preterm neonates may be poorly defined, there is no doubt that parenteral nutrition has transformed the prognosis for newborns when severe congenital or acquired bowel disease precludes enteral feeding.[1] Synthetic crystalline L-amino acid solutions such as Vamin (KabiVitrum), which is based on the amino acid profile of egg protein, have commonly been used as the nitrogen source for parenterally fed children, although they were originally designed for adults. Compared with breast milk (table 1), this results in overprovision of some amino acids (such as phenylalanine) but underprovision of others (such as taurine) that are now considered essential for patients on long term parenteral nutrition.[2]

An additional consideration is that, in contrast to older patients, the most appropriate profile of amino acids administered to newborn infants may not only be different but much less flexible. The need for growth means that the young infant

Table 1 *Daily intake of individual amino acids/kg body weight for infant receiving 2·5 g/kg/day intravenous amino acids or 150 ml/kg/day breast milk*

	Vamin 9 glucose	*Breast milk*	*Vaminolact*
Alanine	107	78	250
Glycine	75	48	83
Proline	288	180	222
Valine	153	130	143
Threonine	107	87	143
Lysine	139	135	222
Serine	267	81	151
Glutamic acid	320	337	282
Leucine	189	180	278
Taurine	0	9	13
Histidine	85	46	83
Arginine	117	73	163
Tyrosine	18	57	20
Phenylalanine	196	72	108
Isoleucine	139	100	123
Cystine	50	37	40
Tryptophan	36	45	56
Methionine	68	28	52
Aspartic acid	146	165	163

requires relatively larger quantities of essential amino acids than an older child or adult. Furthermore, immaturity of enzyme systems in the neonatal liver may reduce the capability for synthesis and catabolism of certain amino acids. For example, liver cystathionase activity is low at full term, and endogenous synthesis of cystine from methionine is limited, making cystine an essential amino acid in the neonate. In addition, although histidine is not considered essential for adults, it seems to be required for normal growth in newborn infants.[3]

We recently showed that plasma amino acid profiles in babies fed with a conventional amino acid source often show increased concentrations of potentially neurotoxic phenylalanine.[4] Others have shared our concern,[5 6] and debate continues over the optimum composition of parenteral feeding solutions.[7] Attempts have been made to design amino acid preparations suited to the particular requirements of newborn infants, in some instances with the aim of producing a plasma amino acid profile during parenteral feeding that is similar to that found in breast fed babies.[8] We have therefore compared amino acid profiles, growth, and nitrogen balance during the use of our usual amino acid solution (Vamin 9 glucose), with a modified formulation containing an amino acid profile more similar to breast milk (Vaminolact, KabiVitrum), in a group of babies admitted to a regional neonatal surgical unit.

Patients and methods

The study was an open, prospective trial, with random allocation of patients to receive either Vamin 9 glucose or Vaminolact. Only patients less than 3 months of age were included. Infants below the 10th weight centile for age, those with heart failure, liver or renal impairment, or known metabolic defects, uncontrolled sepsis or receiving partial enteral feeding were excluded.

GROUP RECEIVING VAMIN 9 GLUCOSE

A group of seven patients received Vamin 9 glucose after operations for the following: small bowel volvulus (n=1), gastroschisis (n=2), small bowel atresia (n=2), and necrotising enterocolitis (n=2). At the start of total parenteral nutrition the median postconceptual age was 38 weeks (range 31–42), median weight 2470 g (range 1470–2910), and median age 16 days (range 11–29).

GROUP RECEIVING VAMINOLACT

A group of seven patients received Vaminolact after operations for gastroschisis (n=3) and necrotising enterocolitis (n=4). At the start of total parenteral nutrition, the median postconceptual age was 39·5 weeks (range 31–43), median weight 2320 g (range 1070–3200) and median age 36 days (range 11–84).

NUTRITIONAL INTAKE

Total parenteral nutrition was prescribed for all patients in accordance with a standard protocol and administered by central venous catheter.[9] Intakes/kg body weight were progressively increased over the first six days of feeding and then maintained at constant amounts as follows: carbohydrate, 8 g, 10 g, 10 g, 12 g, 12 g, 14 g; amino acids, 0·5 g, 1·0 g, 1·5 g, 2·0 g, 2·5 g; fat, 1 g, 2 g, 2 g, 3 g, 3 g, 3·5 g. The maximum daily energy intake was therefore 0·41 MJ/kg. Detailed records of intravenous infusions including additional peripheral infusions were kept so that precise calculation of intakes of fluid, electrolytes, and nutritional components could be made.

ANTHROPOMETRY

Patients were weighed daily to the nearest 10 g using electronic scales (Seca). Additional anthropometric measurements were made on day 0 and day 6 by a single experienced observer; head circumference was measured to the nearest 0·1 cm using a paper tape measure, and triceps and subscapular skinfold thickness to the nearest 0·2 mm with skin calipers (Holtain Ltd).

HAEMATOLOGICAL AND BIOCHEMICAL MONITORING

Infusion of Intralipid was suspended at 5 am each morning until venous blood from a peripheral vein was sampled, between 9 and 10 am. Vamin was constantly infused through the central venous feeding catheter and was not interrupted before the peripheral blood was sampled. Blood was taken on the day after the maximum intake of nutrients was reached (day 0) and then 72 and 144 hours later. In addition to plasma amino acid profile, estimation of sodium, potassium, creatinine, albumin, total bilirubin, calcium, phosphate, magnesium, glucose, and haemoglobin concentrations, full blood count, and alanine aminotransferase and γ glutamyltranspeptidase activities were also carried out. Plasma for amino acid analysis was deproteinised by addition of an equal volume of 3% sulphosalicylic acid immediately after collection. The supernatant was then stored at −20°C until analysis. Plasma concentrations were measured with a Kontron Chromakon 500 amino acid analyser using a lithium buffer system and ninhydrin detection.

NITROGEN BALANCE

All urine, ileostomy, and nasogastric losses were collected during the final 72 hours of the study for subsequent analysis of nitrogen content. During this period patients were nursed on a metabolic bed placed inside an incubator.[10] Urine, nasogastric aspirate, and ileostomy specimens were stored at 4°C until the 72 hour collection was completed. As subjects received no enteral feed during the study, stool losses from the rectum were minimal and were ignored. Specimens were homogenised, total volume measured, and then 20 ml aliquots stored at −20°C until analysis. Nitrogen content was determined using the micro Kjeldahl method[11] and phenol-hypochlorite (Bertholet) reaction.

STATISTICAL ANALYSIS

Comparisons between groups of clinical characteristics, plasma amino acid concentrations, and nitrogen retention were made by the Mann-Whitney U test. Other biochemical observations were compared by Student's *t* test. The mean concentration of each amino acid was calculated after $\log_{10}$ transformation of all the measurements made in one group throughout the study period. Within group changes in variables from day 0 to day 6 were compared using a paired *t* test. A probability of <0·05 was accepted as significant.

Approval for the study was obtained from the research ethical committee of the Central Birmingham Health Authority. Informed parental consent was obtained before starting total parenteral nutrition.

Results

There were no significant differences between patients given Vamin 9 glucose and those given Vaminolact in age in days, postconceptual age, weight at the start of total parenteral nutrition, or intakes of fluid, nitrogen, carbohydrate, or energy. No adverse reactions to feeding solutions were seen in either group and no patients were septic during the period of study.

ANTHROPOMETRY

Each group showed a significant increase in weight, head circumference, and triceps skinfold thickness over the six day study period (p<0·05) (table 2). Increases in subscapular skinfold thickness were not significant. Median weight gain was 37 g/day (range 23–60 g) in the Vamin 9 glucose group, and 32 g (10–38 g) in the Vaminolact patients (p=0·26). No significant differences were found in changes in other anthropometric variables between groups.

NITROGEN BALANCE

The median nitrogen intake for Vamin 9 glucose

Table 2 *Growth during study period*

	Vamin 9 glucose	*Vaminolact*
Median daily increase in weight (range)	37 g (23–60)	32 g (10–38)
Median increase in triceps skinfold thickness during six day study (range)	0·6 mm (0–1·6)	0·4 mm (0–[illegible]·6)
Median increase in subscapular skinfold thickness during six day study (range)	0·4 mm (0·2–3·0)	0·4 mm (0–[illegible]·4)
Median increase in head circumference during six day study (range)	0·6 cm (0–1·2)	0·4 cm (0–0·9)

Table 3 *72 Hour nitrogen balance*

Weight (g)	*Nitrogen intake (mg)*	*Urinary nitrogen loss (mg)*	*Nasogastric nitrogen loss (mg)*	*Ileostomy nitrogen loss (mg)*	*Nitrogen retention (%)*
		Group receiving Vamin 9 glucose			
2550	2340	228	118	0	85
1470	1215	225	7	17	80
2470	2175	330	128	0	79
2910	2262	490	66	0	75
2000	1800	525	23	0	70
1930	1653	460	0	0	72
2630	1872	904	0	0	52
		Group receiving Vaminolact			
2550	2340	343	0	0	85
3200	2304	453	0	0	80
2740	2187	440	14	0	79
2320	1863	347	0	139	74
1070	957	278	4	0	70
1440	1210	404	0	171	52
2180	1584	875	17	0	44

Table 4 *Mean (range) plasma amino acid concentrations (μmol/l) in patients receiving Vamin 9 glucose and Vaminolact*

Amino acid	*Target range*[12]	*Vamin 9 glucose*	*Vaminolact*	*p Value*
Alanine	125–647	251 (87–872)	305 (125–509)	0·66
Glycine	77–376	460 (232–937)	448 (220–701)	0·68
Proline	83–319	312 (85–458)	153 (171–356)	0·03
Valine	88–222	222 (69–331)	152 (106–237)	0·0002
Threonine	70–197	248 (70–440)	367 (145–699)	0·003
Lysine	80–232	82 (43–195)	206 (71–388)	0·0001
Serine	0–326	268 (86–689)	218 (110–579)	0·15
Glutamic acid	24–243	78 (20–764)	98 (40–550)	0·8
Leucine	53–169	94 (49–144)	108 (47–226)	0·03
Taurine	1–167	48 (24–186)	88 (52–245)	0·01
Histidine	34–119	92 (50–153)	99 (60–145)	0·5
Arginine	42–148	32 (16–78)	51 (21–148)	0·0005
Tyrosine	38–119	146 (43–572)	28 (5–69)	0·0004
Phenylalanine	22–70	192 (55–800)	80 (47–167)	0·0028
Isoleucine	27–90	55 (11–124)	50 (22–100)	0·029
Cystine/cysteine	99–208	52 (25–108)	38 (10–70)	0·047
Tryptophan	19–100	30 (20–42)	49 (26–69)	0·0001
Methionine	22–50	39 (12–69)	25 (13–40)	0·0001
Aspartic acid	5–51	19 (7–73)	23 (12–72)	0·6

patients was 857 mg/kg/72 hours (range 712–918) and for Vaminolact patients 803 mg/kg/72 hours (range 720–916). The median nitrogen retention calculated from the three day balance study was 75% (range 52–85) for Vamin 9 glucose and 74% (range 44–85) for Vaminolact (table 3). The nitrogen intakes and retentions were not significantly different between the groups.

HAEMATOLOGICAL AND BIOCHEMICAL MEASUREMENTS

No haematological or biochemical measurements changed significantly in either group over the six day study period. In particular, no patients showed biochemical evidence of liver dysfunction. Other than plasma amino acid concentrations, no haematological or biochemical measurements in the two groups were significantly different.

PLASMA AMINO ACID PROFILES

For each group, the mean and range of plasma concentrations for individual amino acids are shown in table 4; the quoted target ranges represent the 95% confidence interval derived from amino acid profiles in breast fed infants.[12] Few amino acids were consistently found at concentrations within the target range. Phenylalanine concentrations in subjects fed Vaminolact were significantly lower than in the Vamin 9 group (p=0·0028) as were concentrations of tyrosine (p=0·0004). In the group given Vaminolact, 80% of the tyrosine values fell below, and 20% within, the target range, while in the Vamin 9 group 58% were above the upper target range and none below. Total cystine/cysteine concentrations always fell below the lower target range in the Vaminolact group, and only one value in the Vamin 9 group was above this. In the subjects fed Vaminolact, the mean concentrations of all amino acids except threonine, lysine, histidine, and cystine were closer to the reference means than in Vamin 9 glucose fed infants.[12]

Discussion

The results of this study indicate that the use of a neonatally adapted amino acid source leads to a reduction in the derangement of plasma amino acid profiles, while growth and nitrogen retention are unchanged. Previous studies have shown that plasma phenylalanine is higher in parenterally fed babies than in those receiving enteral feeds,[13 14] and particular attention has been drawn to the observation that high phenylalanine intakes in neonates receiving Vamin 9 glucose may be associated with potentially neurotoxic plasma phenylalanine concentrations.[4–6] Two of the seven babies given Vamin 9 glucose during this study had plasma phenylalanine concentrations above 600 μmol/l, which would be considered unacceptably high in a child with treated phenylketonuria.[15] Phenylalanine concentrations in children receiving Vaminolact were much lower (p=0·0028), although a high proportion (68%) were still above the upper limit of the target range.

There are several reasons why plasma amino acid profiles in patients given Vaminolact still differ from those in breast fed infants. An intravenous amino acid intake of 2·5 g/kg/day is 25% higher than with breast milk of average composition. Furthermore, plasma amino acid profiles in breast fed infants reflect modification of relative amino acid concentrations during small intestinal absorption and subsequent passage of portal blood through the liver. That such modification occurs is one reason why attempts to reproduce those plasma amino acid profiles found in breast fed infants exactly in parenterally fed infants are probably misguided.

The mean tyrosine concentration in infants fed Vamin 9 glucose was also high compared with that reported in breast fed infants,[12] and tyrosine concentrations were significantly higher than those found in the Vaminolact group (p=0·0004). This too is a cause for concern, as it has been suggested that raised tyrosine concentrations may be related to long term developmental impairment.[16] The raised tyrosine concentrations probably reflect phenylalanine catabolism, as the intake of tyrosine with both Vamin 9 glucose and Vaminolact is similar. The low mean concentration of tyrosine found in the Vaminolact group suggests possible underprovision of this amino acid, which is said to be 'semi-essential' in the very low birthweight infant. This might pose problems in long term parenteral nutrition, although the consequences of a low tyrosine intake are unknown.

The increased threonine concentrations with Vaminolact reflect the fact that intake of threonine is about 50% higher than in the Vamin 9 glucose or breast fed infants, but does not exceed the recommended maximum for preterm infants.[17] Although concentrations of cystine/cysteine were low, clinical studies have shown that cystine deficient diets have no apparent adverse effects on growth or nitrogen balance in preterm infants and the precise requirements for cystine remain unknown.[18 19]

The implications of the hyperphenylalaninaemia observed in the Vamin 9 glucose group are difficult to assess. Recent evidence suggests that appreciable intellectual impairment may occur in patients with phenylketonuria even when plasma phenylalanine concentrations have been kept as low as 600 μmol/l.[20] Although courses of parenteral nutrition will in most cases be measured in weeks rather than months, the neonatal brain is undergoing a period of rapid growth and development and might therefore be particularly sensitive to injury. Studies in animals have shown that even transient rises of plasma phenylalanine may be associated with enduring behavioural changes[21] and poor brain growth.[22] High concentrations of phenylalanine during parenteral nutrition must therefore be regarded as potentially harmful. The widespread experience with Vamin 9 glucose suggests, however, that the risk of clinically apparent neurological injury in the short term is extremely small.

Amino acid solutions such as Vaminolact that are specifically designed to meet the needs of infants are likely to lessen the risk of neurotoxicity resulting from amino acid imbalance, and we now use a neonatally adapted formula (Vamin Infant*) in patients less than 6 months of age. We reject the view that measurement of plasma amino acid concentrations during parenteral nutrition is unnecessary,[23] and recommend weekly monitoring —at least of phenylalanine—in patients up to 6 months of age who are receiving an unmodified, high phenylalanine, amino acid solution.

*Vamin Infant is available in the United Kingdom and is identical to Vaminolact except that it does not contain taurine. Vaminolact does not yet have a product licence in the United Kingdom.

We thank the nursing staff of Ward PB2 whose skills, help, and enthusiasm made this study possible, and Dr R Wild of KabiVitrum for supplying the Vaminolact. We also thank the department of medical physics, Birmingham Maternity Hospital, and the Central Birmingham Health Authority workshops for constructing the metabolic bed, the plans for which were kindly provided by Dr MG Coulthard.

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References

[1] Dorney SFA, Ament ME, Berquist WE, Vorgas JH, Hassall E. Improved survival in very short small bowel of infancy with use of long-term parenteral nutrition. *J Pediatr* 1985;**107:**521–5.

[2] Geggel HS, Ament ME, Heckenlively JR, Martin DA, Kopple JD. Nutritional requirement for taurine in patients receiving long-term parenteral nutrition. *N Engl J Med* 1985;**312:**142–6.

[3] Snyderman SE, Boyer A, Roitman E, Holt LE, Prose PH. The histidine requirements of infants. *Pediatrics* 1963;**31:**786–801.

[4] Puntis JWL, Edwards MA, Green A, Morgan I, Booth IW, Ball PA. Hyperphenylalanaemia in parenterally fed newborn babies. *Lancet* 1986;**ii:**1105–6.

[5] Walker V, Hall MA, Bulusu S, Allan A. Hyperphenylalanaemia in parenterally fed newborn babies. *Lancet* 1986;**ii:**1284.

[6] Evans SJ, Wynne-Williams TCJE, Russell CA, Fairbrother A. Hyperphenylalaninaemia in parenterally fed newborn babies. *Lancet* 1986;**ii:**1404–5.

[7] Lemons JA, Neal P, Ernst J. Nitrogen sources for parenteral nutrition in the newborn infant. *Clin Perinatol* 1986;**13:**91–109.

[8] Heird WC, Dell RB, Helms RA, *et al.* Amino acid mixture designed to maintain normal plasma amino acid patterns in infants and children requiring parenteral nutrition. *Pediatrics* 1987;**79:**401–8.

[9] Hughes CA. Parenteral nutrition. In: Insley J, Wood B, eds. *A paediatric vade mecum.* London: Lloyd-Luke, 1984:60–7.

[10] Coulthard MG. Device for continuous urine collection in the newborn. *Arch Dis Child* 1982;**57:**322.

[11] Fleck A, Munro HN. The determination of organic nitrogen in biological materials. *Clin Chim Acta* 1965;**11:**2–12.

[12] Wu PYK, Edwards N, Storm MC. Plasma amino acid pattern in normal breast fed infants. *J Pediatr* 1986;**109:**347–9.

[13] Hume R, Giles MM, Cockburn F. Amino acid analysis in parenteral nutrition of the newborn. In: Rattenbury JM, ed. *Amino acid analysis.* Chichester: Horwood, 1981:131–8.

[14] Rigo J, Senterre J. Parenteral nutrition in the very low birth weight infant. In: Krechmer N, Minkowski A, eds. *Nutritional adaptation of the gastrointestinal tract of the newborn*. New York: Nestle, Vevey/Raven Press, 1983:191–207.

[15] Tourian A, Sidbury JB. Phenylketonuria. In: Stanbury JB, Wyngaarden JB, Fredrickson DS, eds. *The metabolic basis of inherited disease*. New York: McGraw Hill, 1982:270–86.

[16] Mamunes P, Prince PE, Thornton NH, Hunt PA, Hitchcock ES. Intellectual deficits after transient tyrosinaemia in the term neonate. *Pediatrics* 1976;**57**:675–80.

[17] Rigo J, Senterre J. Optimal threonine intake for preterm infants fed on oral or parenteral nutrition. *JPEN* 1979;**4**:15–7.

[18] Malloy MH, Rassin DK, Richardson CJ. Total parenteral nutrition in sick pre-term infants: Effects of cysteine supplementation with nitrogen intakes of 240 and 400 mg/kg/day. *J Pediatr Gastroenterol Nutr* 1984;**3**:239–44.

[19] Zlotkin SH, Bryan MH, Anderson GH. Cysteine supplementation to cysteine free intravenous feeding regimens in newborn infants. *Am J Clin Nutr* 1981;**34**:914–23.

[20] Wolff O, Smith I, Beasley M. Quelques limites de la classification et de la nomenclature des hyperphenylalaninemies. *Arch Fr Pediatr* 1987;**44**:635–8.

[21] Andersen AE, Guroff G. Enduring behavioural changes in rats with experimental phenylketonuria. *Proc Natl Acad Sci USA* 1972;**69**:863–8.

[22] Prensky AL, Fishman MA, Deftari B. Recovery of rat brain from a brief hyperphenylalaninaemic insult early in development. *Brain Res* 1974;**73**:51–6.

[23] Kerner JA. Monitoring of pediatric parenteral nutrition in the hospital and at home. In: Lebenthal E, ed. *Total parenteral nutrition*. New York, Raven Press 1986:231–44.

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Growth velocity and stunting in rural Nepal

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SUMMARY In a community based study, height and weight increments of 441 Nepali children aged 0–6 years were measured before harvest and six months later and compared with centile standards derived from American children. Low mean growth velocities for height were found only in children under 2 years of age, and for weight during the first 18 months. The mean height for age standard deviation score for the 12–23 months age group was already −2·8 at first measurement. The effect of the initial thinness of the child on subsequent height and weight velocity was reciprocal: thin children seemed to catch up weight at the expense of height.

These results suggest that stunting is caused largely by a reduced growth velocity during the nutrition dependent infantile phase of growth, with some additional impairment and delay in onset of the early childhood phase of growth hormone dependent growth, especially in thin children. Nutritional interventions after the second year of life are unlikely to alter the prevalence of linear growth retardation in poor communities. Growth velocity may be more useful than static anthropometry to assess the impact of such interventions.

The aetiology of linear growth retardation in poor communities remains unclear. Ethnic differences are less important than environmental influences,[1 2] although there is variation in the ability of different genotypes to cope with hardship.[3] Prevalence rates of stunting are associated with variables such as the age and sex of the child, the frequency of episodes of infection and socioeconomic indicators of household poverty.[4–8] The importance of seasonal variation in growth rates has also been noted in longitudinal surveys.[8 9] These studies have analysed static height for age values, whereas growth velocity measurements are more valuable than 'distance charts' in the assessment of growth, which is a dynamic process.[10] Growth velocity is independent of the height achieved by a child so it is a sensitive indicator of good or bad health regardless of previous growth delay. The normal linear growth curve has recently been described in terms of an infancy-childhood-puberty model,[11] which suggests that linear growth comprises three additive, partly superimposed mathematical components, an infancy component, which represents the postnata contribution of nutrition dependent fetal growth, a childhood component, which is controlled by growth hormone,[12] and a puberty component, which is dependent on sex steroids and growth hormone.

The aim of the present study was to measure growth velocities of children during a six months period after harvest when growth rates would be expected to be maximal in a community where stunting is common, and to consider the relative contributions of infancy compared with childhood growth retardation to the process of stunting.

Subjects and methods

BAGLUNG DISTRICT

Baglung District covers 2153 square km of a remote rural area in western Nepal where there are no motorable roads. To the north it is bounded by the Himalayan mountains and to the south by deep river valleys and basins. Most of the population (1985 estimate: 232 000) live in the middle hill region below 2500 m where they produce on terraced hills rice, maize, millet, and wheat in a monsoon climate. Nearly all the households depend on subsistence agriculture, and Baglung is a food deficit area with an average annual household grain production of about eight months. The district is divided into 62 geopolitical subdivisions called panchayats.

DESCRIPTION OF SURVEY

The survey covered all 349 households with children less than 6 years of age in a single panchayat, Resa, situated about four hours walk from the district capital, Kalika. It was planned after discussions with the pradhan panch (mayor) of Resa, representatives from each of the nine wards in the panchayat, and staff from the health post in Resa and the Save the Children Fund clinic in Kalika.

All houses with children under 6 years were visited on two occasions, during August 1985 (monsoon) and six months later during February 1986 (dry season). Wards were surveyed sequentially by teams comprising one health post person, one Save the Children Fund health worker, and a local man who could identify the houses.

Before starting the survey staff were trained in interview techniques; given practice with the completion of questionnaires at houses near the Save the Children Fund clinic in Baglung; and taught how to measure weights and heights using the Salter scales, the baby measuring cradle, and the height frame. Weights were recorded to the nearest 0·1 kg and heights to the nearest 0·1 cm. The head of the household and the children's parents were told the purpose of the survey and asked for their cooperation. If parents or children were away, they were visited again the next day. The team interviewed the household head or one of the parents; recorded household, ethnic group, and health related data on a precoded questionnaire; and examined and measured all children under 6 years of age.

The survey teams calculated the age of each child to the nearest complete month by checking the month and year of birth with two adults, and by using a calendar table that eliminated the local custom of overestimating age by one month or one year.

DATA ANALYSIS

Age distribution

The age and sex distribution of the child population surveyed was checked against nutrition surveys conducted nationwide in 1975 and a Baglung nutrition survey in 1979. The proportion of children aged 60–71 months in both Baglung survey samples was slightly lower than expected.

Nutritional measurements

The height and weight measurements recorded at each visit were expressed as the percentage of a reference median and as standard deviation scores for height for age, weight for height, and weight for age using a statistical programme based on the National Centre for Health Statistics standards.[13] Differences between weights and heights at each visit allowed calculation of weight and height increments over the six month period between August and February when, being the period after the harvest, nutrition would be expected to be optimal (or nearly so) in this community. These increments were compared with centiles and standards drawn from the Fels study in the USA.[14]

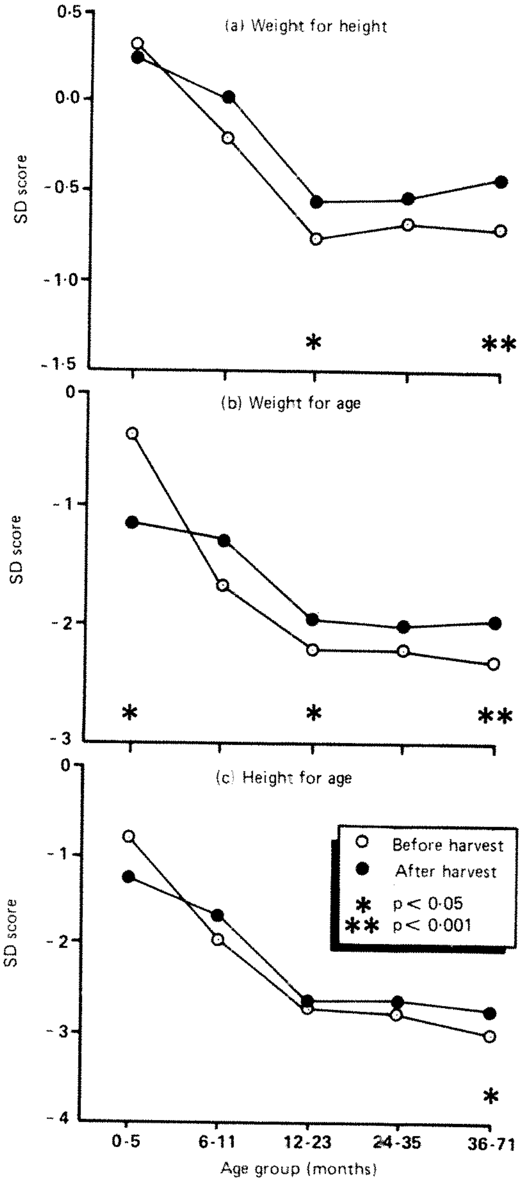

Fig 1 *The mean (SD) scores of static nutritional measurements (a) weight for height, (b) weight for age, and (c) height for age, measured in the surveys before and after the harvest for different age groups. Significant differences for each age group are shown (unpaired Student's t test).*

Data handling and statistics

The questionnaire data were precoded where possible for ease and accuracy of collection. The data were double checked for obvious errors of translation before entry into the computer database. Statistical calculations were made using the Apple Statview programme.

Results

A total of 546 children aged 0–71 months were seen before (August 1985), and 479 seen after (February 1986) the harvest; the latter group included newborns from the intervening period. Growth increments were obtained in 441 children (81%). Using the Waterlow classification of nutritional status,[4] 296 children (54%) were stunted (< 90% of the height for age reference median) and 17 (3·1%) were wasted (< 80% of the weight for height reference median) in the survey before the harvest; 228 (51%) were stunted and nine (1·9%) wasted in the survey after the harvest. Stunting prevalences were highest before the harvest for all age groups, and among the poorest households and lowest caste children; they rose sharply between 12 and 23 months of age.

Fig 1 shows the mean standard deviation scores (SD score) for static nutritional measurements (weight/height, weight/age, height/age) for different age groups at each survey visit. There were significant seasonal differences for weight/height and weight/age scores overall (children were thinner before the harvest), but height/age scores showed no

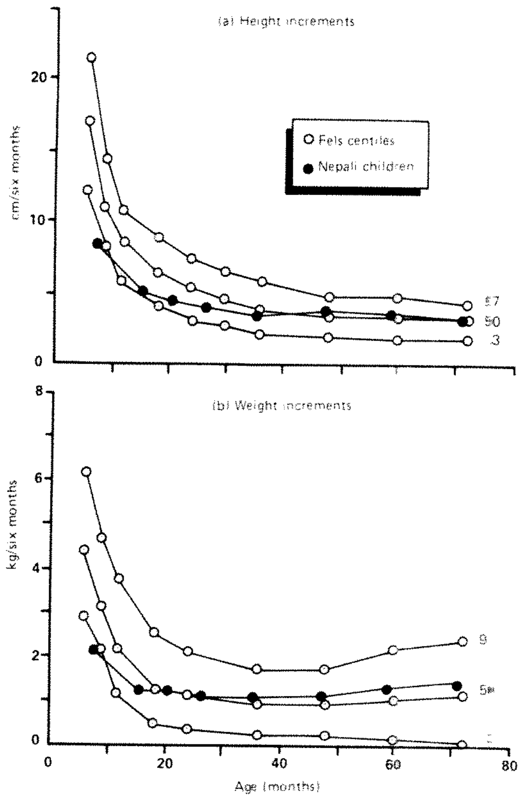

Fig 2 *Mean height and weight increment curves for Nepali children compared with increment centiles for American children in the Fels study. (The Fels velocity standards were also established using an incremental time interval of six months.[14])*

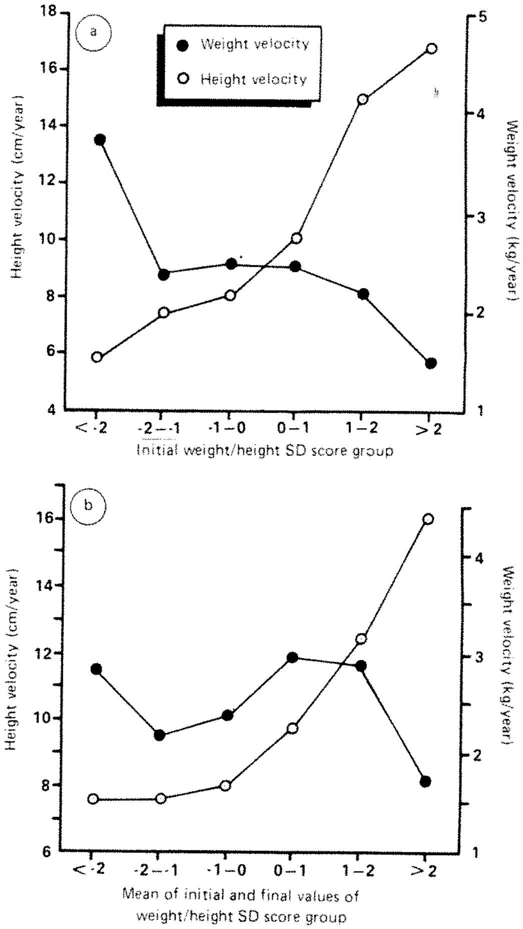

Fig 3 *(a) The effect of a child's initial nutritional state (classified by the initial weight for height SD score group) on subsequent mean weight and height velocity in the period after the harvest. (b) Oldham's plot for correction of the phenomenon of regression to the mean, using the mean of the initial and final values of weight/height SD score group as a measure of nutritional state.*

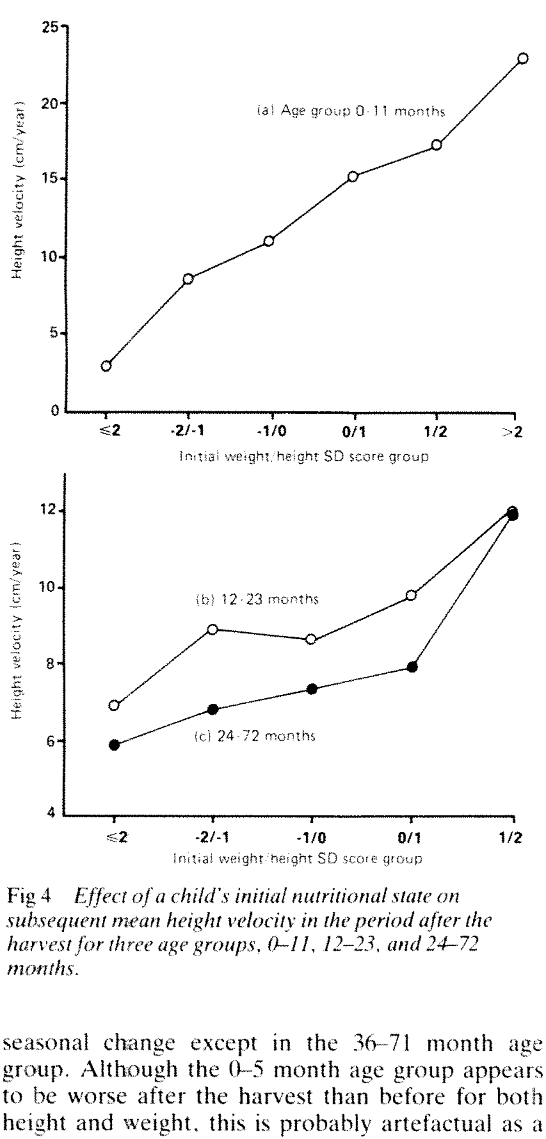

Fig 4 *Effect of a child's initial nutritional state on subsequent mean height velocity in the period after the harvest for three age groups, 0–11, 12–23, and 24–72 months.*

seasonal change except in the 36–71 month age group. Although the 0–5 month age group appears to be worse after the harvest than before for both height and weight, this is probably artefactual as a result of the relatively low numbers of infants aged 0–5 months measured after the harvest.

The mean growth increment curves of the survey children by age group compared with incremental centiles established in American children are shown in fig 2. If values below the 25th centile are considered arbitrarily to be abnormal, then height increments were abnormally low until 24 months and weight increments until 18 months of age. Increments returned to near the 50th centile by 22 months for weight and 36 months for height.

The influence of the initial state of the child (assessed by weight/height SD score before the harvest) on subsequent height and weight velocity is shown in fig 3(a). Analysis of raw data showed a highly significant relationship between the initial weight/height SD score and height velocity (line of best fit $y=9{\cdot}36+2{\cdot}28x+0{\cdot}44x^2$, $p<0{\cdot}001$) and there was an inverse but weaker association with weight velocity (line of best fit $y=2{\cdot}36-0{\cdot}13x+0{\cdot}14x^2-0{\cdot}05x^3$, $p<0{\cdot}005$).

This association between initial weight/height SD score and growth velocity is complicated however by two potential sources of error, regression to the mean and the uneven distribution of weight/height scores with age, high values of weight for height tending to occur in the youngest age group when both height and weight velocity are highest. The error introduced into these relationships by the phenomenon of regression to the mean was corrected using Oldham's method[15] of plotting the velocities against the average of initial and final values of weight/height SD scores, as shown in fig 3(b), which still shows a significant relationship between the thinness of the child and catch up height velocity ($y=8{\cdot}98+1{\cdot}71x+0{\cdot}48x^2$, $p<0{\cdot}001$); fig 4 shows the same relationship for each of the age groups 0–11 months, 12–23 months, and 24–72 months of age, again showing this association, especially in the first two years of life.

Discussion

Although growth velocity measurement (like most methods of growth monitoring) is of limited clinical value in primary care in developing countries, it has advantages as an epidemiological tool when compared with static anthropometric measurements. Velocity is more time specific than a distance measure like height for age: a normal velocity indicates current good health in children who are stunted from previous causes, and a reduced growth velocity suggests poor health even in children whose growth has not yet faltered appreciably on a distance chart.

Chen has suggested a need for better, simpler and more sensitive epidemiological markers than infant or child mortality rates to assess the impact of primary health care initiatives in developing countries.[16] Growth velocity measurements fufil these criteria. With standardised centiles for growth increments now available, they can be used to identify not only vulnerable groups but also vulnerable time periods in a particular community.

The results of this study confirm a previous finding that stunting has an early onset in poor communities.[9] The maximal impairment of height velocity seems to occur during the latter postnatal stage of the nutritional component of linear growth, although increments in the first 6 months of life cannot be assessed from these data. Waterlow reviewed surveys in similar communities where breastfeeding is widespread, and showed that growth rates (assessed only by weight velocity) falter from 3 months of age compared with those of British children,[17] although the measurement of velocity at this time is difficult. Further, the onset of the growth hormone dependent childhood component of growth may be delayed compared with children in developed countries, in whom it begins between 6–12 months of age.[11] Karlberg *et al* showed an average delay in onset of five months for children from an urban community in Lahore, Pakistan.[18]

The most vulnerable period for impairment of growth rates is between weaning and 24 months. Mean growth velocities after this age in Nepali children were close to the 50th centile for American children. This observation is important if, for economic reasons, nutritional measures are to be targeted selectively as part of a national or international health policy. Overall, nutritional and health interventions after the second year of life are unlikely to alter appreciably the prevalence of linear growth retardation. Seasonally adjusted mean growth velocity rates may, however, be a good indicator of the impact of such interventions in this age group.

Our community based results also show the clear effect of the initial nutritional state of the child on subsequent height velocity, and help to clarify the complex relationship between wasting and stunting. Thin children gained weight faster than their normal peers but at the expense of their height velocity. This supports the hypothesis that the frequency and duration of preceding wasting episodes influence the likelihood and degree of stunting. In Nepal and most developing countries prevalence rates for wasting are highest in the 12–23 month age group and wasting probably plays a large part in the reduction in mean height velocity at this time.

Walker and Golden have shown in a hospital based study that malnourished children need to reach a critical threshold of 85% weight/height before catch up linear growth will occur.[19] The mechanism of growth failure in patients with protein energy malnutrition appears to be a deficiency in the generation of insulin[20] and somatomedins,[21] especially insulin like growth factor 1, which mediates the growth of epiphyseal cartilage.

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References

[1] Habicht J, Martorell R, Yarborough C, *et al.* Height and weight standards for preschool children. How relevant are ethnic differences in growth potential? *Lancet* 1974;**i:**611–5.

[2] Farquharson SM. Growth patterns and nutrition in Nepali children. *Arch Dis Child* 1976;**51:**3–12.

[3] Tanner JM. Population differences in body size, shape, and growth rate. *Arch Dis Child* 1976;**51:**1–2.

[4] Waterlow JC. Classification and definition of protein-calorie malnutrition. *Br Med J* 1972;**iii:**566–9.

[5] Nabarro D, Howard P, Cassels C, Pant M, Wijga A, Padfield N. The importance of infections and environmental factors as possible determinants of growth retardation in children. In: Waterlow J, ed. *Linear growth retardation in less developed countries.* Nestle Nutrition Workshop Series, Vol 14. New York: Raven Press, 1988:165–83.

[6] Howard PF. Nutritional and socio-economic status of children in Western Nepal—a baseline survey as a guide to planning. London: London School of Hygiene and Tropical Medicine, 1984. (Thesis.)

[7] Cutting Wam, Elton RA, Campbell JL, *et al.* Stunting in African children. *Arch Dis Child* 1987;**62:**508–9.

[8] Brown KH, Black RE, Becker S. Seasonal changes in nutritional status and the prevalence of malnutrition in a longitudinal study of young children in rural Bangladesh. *Am J Clin Nutr* 1982;**36:**303–13.

[9] McGregor IA, Rahman AK, Thompson B, *et al.* The growth of young children in a Gambian village. *Trans R Soc Trop Med Hyg* 1968;**62:**341–52.

[10] Brook CGD, Hindmarsh PC, Healy MJR. A better way to detect growth failure. *Br Med J* 1986;**293:**1196.

[11] Karlberg J, Engstrom I, Karlberg P, Fryer JG. Analysis of linear growth using a mathematical model. *Acta Paediatr Scand* 1987;**76:**478–88.

[12] Hindmarsh P, Smith PJ, Brook CGD, Matthews DR. The relationship between height velocity and growth hormone secretion in short pre-pubertal children. *Clin Endocrinol* 1987; **27:**581–91.

[13] World Health Organisation. *Measuring change in nutritional status.* Geneva: WHO, 1983.

[14] Baumgartner RN, Roche AF, Himes JH. Incremental growth tables: supplementary to previously published charts. *Am J Clin Nutr* 1986;**43:**711–22.

[15] Hayes RJ. Methods for assessing whether change depends on initial value. *Stat Med* 1988;**7:**915–27.

[16] Chen LC. Primary health care in developing countries: overcoming operational, technical and social barriers. *Lancet* 1986; **ii:**1260–4.

[17] Waterlow JC, Ashworth A, Griffiths M. Faltering in infant growth in less-developed countries. *Lancet* 1980;**ii:**1176–8.

[18] Karlberg J, Jalil F, Lindblad BS. Longitudinal analysis of infantile growth in an urban area of Lahore, Pakistan. In: Karlberg J, ed. *Modelling of human growth.* Goteborg: University of Goteborg, 1987. (PhD thesis.)

[19] Walker SP, Golden MHN. Growth in length of children recovering from severe malnutrition. *European Journal of Clinical Nutrition* 1988;**42:**395–404.

[20] Hindmarsh PC, Matthews DR, De Silvio L, *et al.* Relationship between height velocity and fasting insulin secretion in children. *Arch Dis Child* 1988;**63:**665–6.

[21] Suliman AT, Hassan AEH, Aref MK, *et al.* Serum insulin like growth factors 1 and 2 concentrations and growth hormone and insulin responses to arginine infusion in children with protein energy malnutrition before and after nutritional rehabilitation. *Pediatr Res* 1986;**20:**1122–30.

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Growth evaluation: parent and child specific height standards

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SUMMARY Data on the growth of 1063 children and their parents were analysed. Of the variation in height at prepuberty about 20%, and of the final height 30–46%, were explained by the variation in parental heights; the children's own height at the age of 1·0 year increased the proportion explained to half. Two equations were developed for increasing the accuracy of the evaluation of growth. One defines parent specific mean height standard deviation score, and the other includes the parents' heights and the child's height at the age of 1·0 year and defines expected standard deviation score for height. These equations are easy to use if they are converted to nomograms.

The accuracy of the evaluation of growth could be improved by the use of parent and child specific standards instead of population standards. Tanner *et al* published parent specific standards,[1] but their use requires a special chart and their validity for other populations is unknown.

We have analysed the longitudinal growth and maturation of 1063 Finnish children and their parents, and present practical new methods for obtaining parent specific height standard deviation scores, and for including the child's height at the age of 1·0 year in the assessment of growth.

Subjects and methods

All heights and weights recorded in child health

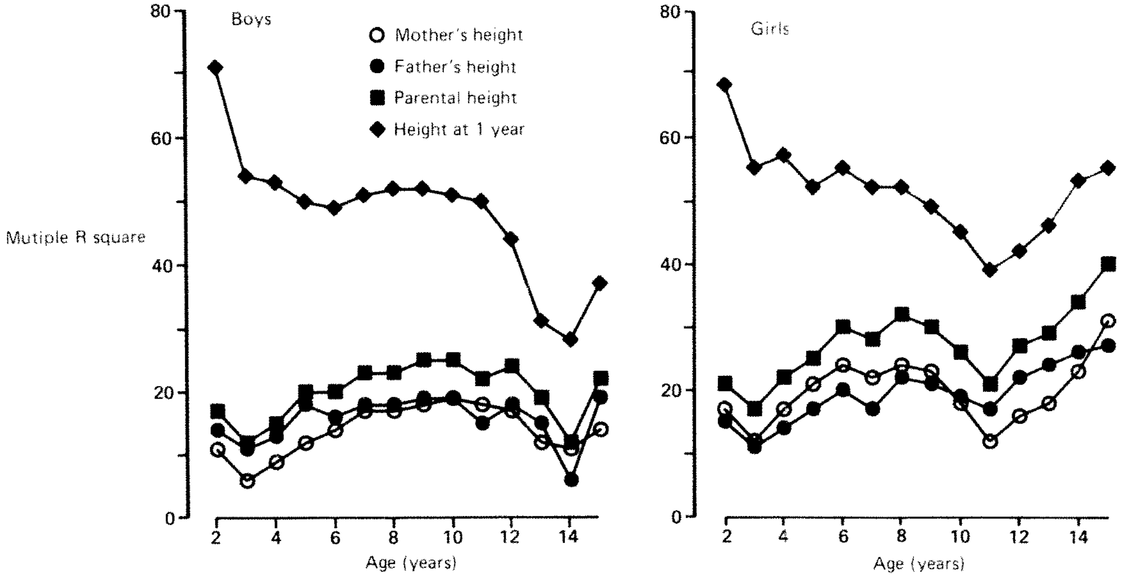

Fig 1 *The proportion of the variation (R square in multiple regression) in height at different ages explained by the heights of parents and children's own heights at the age of 1·0 year for 231 boys and 231 girls.*

centres and schools were collected from birth (in 1959–1961) until 1978 for 544 healthy boys and 519 girls: 412 boys and 351 girls from the city of Helsinki and 132 boys and 168 girls from eastern Finland. The sample is representative of the whole social class structure. The population of Helsinki is a mixed one, originating from all areas of Finland. The boys were an unselected group of military conscripts; the girls were secondary school graduates. Eastern Finland was selected for the second sample as the most likely area to show any difference from the mixed population. Because there was no significant difference between the two samples they were pooled and considered as being representative of the whole population.

We evaluated height as standard deviation scores, using linear interpolation to obtain the height at each full year from actual measurements made within half a year. The latest height available, recorded after the age of 17·0 for girls and 18·0 years for boys, was regarded as the final height, provided that the increment during the preceding year had been less than 1·0 cm. The age just before height velocity began to increase ('take off')[2] and the age at the peak height velocity during puberty were determined individually by smoothing individual growth velocity data by a cubic square spline function.[3]

From a questionnaire we ascertained the heights of the parents, the ages at menarche of the girls and their mothers, and the timing of growth and maturation in the fathers (early, average, or late growth spurt compared with peers). Virtually all men have their height at the age of 20 recorded in their military passport. If no accurate height measurement was on record the parents were asked to have their heights measured at the local health centre.

For statistical evaluation we used simple and multiple linear regression analysis.[4]

Results

We calculated the proportion of the variation by

Table 1 *Percentages of the variations in final height in 231 boys and 231 girls (R square in multiple regression analysis) explained by variation in the child's own heights at full years 0 to 5 and parental heights*

	Boys	*Girls*
Birth height	10	14
Height at 1 year	35	36
Height at 2 years	38	52
Height at 3 years	33	45
Height at 4 years	50	54
Height at 5 years	57	63
Father's height	22	32
Mother's height	24	35
Both parents' heights	30	46
Both parents' heights plus child's birth height	35	52
Both parents' heights plus child's height at 1·0 year	53	57
Both parents' heights plus child's height at 5·0 years	60	72

Table 3 *The differences (cm) between the final height measured and the adult cm values corresponding to the height standard deviation scores determined by the three equations (table 2)*

		Difference	
	No	*Mean*	*SD*
Boys:			
SDS 1	474	−0·2	5·3
SDS 2	474	−0·8	5·3
SDS 3	335	0·4	4·3
Girls:			
SDS 1	466	0·1	4·8
SDS 2	466	−0·4	4·9
SDS 3	301	−0·2	3·7

Table 2 *Equations for determination of parent specific mean height standard deviation scores (SDS 1 and SDS 2) and parent and child specific expected height standard deviation scores (SDS 3). The equations for SDS 1 and SDS 3 are based on the present data for 335 boys and 301 girls. The equation for SDS 2 is: midparental height minus population mean midparental height, divided by 10 (the product of 5 and 2, where 5 is the approximate value of height SD for adults.[5] H=height (cm), H 1·0=height at age 1·0 year. Values of constants are given below.*

$SDS_1 = X_1 \times H \text{ of father} + Y_1 \times H \text{ of mother} + Z_1$

$SDS_2 = ((H \text{ of father} + H \text{ of mother}) \div 2 - 170) \div 10.$

$SDS_3 = X_3 \times H \text{ of father} + Y_3 \times H \text{ of mother} + V \times H\ 1{\cdot}0 + Z_3$

	X_1	Y_1	Z_1	X_3	Y_3	V	Z_3
Boys	0·0607	0·0638	−21·0	0·0423	0·0526	0·172	−29·0
Girls	0·0619	0·0765	−23·3	0·0420	0·0552	0·170	−29·2
Both	0·0611	0·0703	−22·1	0·0423	0·0542	0·166	−28·9

multiple regression analysis (%, multiple R square) in the heights of children explained by the variation in the heights of their parents. Before puberty, at the ages of 2·0 to 13·0 years for boys and 2·0 to 11·0 years in girls, 6–24% of the variation in the children's height was explained by the variation in height of the fathers or the mothers, and 15–32% by the variation in height of both parents (fig 1). These proportions were higher for girls than for boys. Inclusion in the analysis of the children's own heights at the age of 1·0 year improved the proportions explained (fig 1).

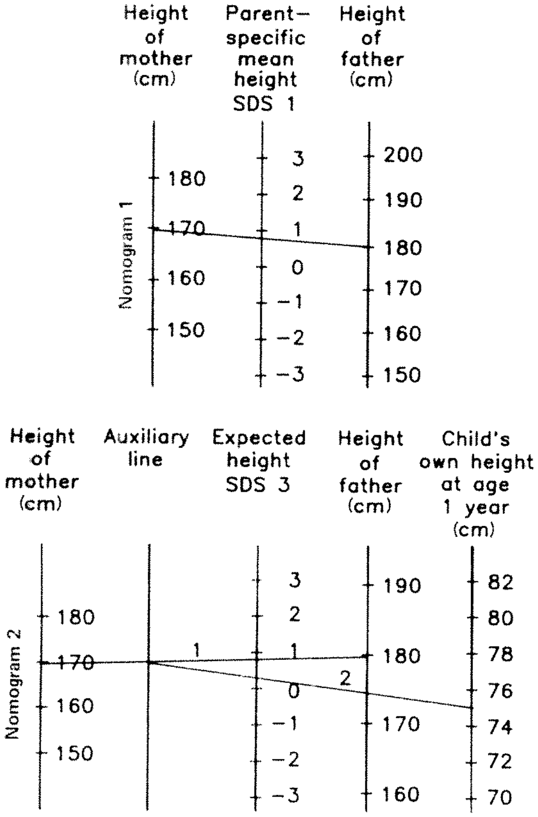

Fig 2 *Nomograms for prediction of height (for equations see table 2). Nomogram 1: draw a line to connect the heights of mother and father. The cut off point indicates the parent specific mean height standard deviation score (SDS 1). Nomogram 2: first draw line 1 to connect the heights of mother and father. Then draw line 2 to connect the child's own height at the age of 1·0 year with the cut off point of line 1 and the auxiliary line. The cut off point of line 2 indicates the child specific expected height standard deviation score (SDS 3). Example: if the height of the father is 180 cm, the height of the mother 170 cm, and the child's own height at age 1·0 year 75 cm, then parent specific mean height standard deviation score is +0·8 and child specific expected height standard deviation score 3 is +0·4.*

We made a similar calculation for the proportion of the variation in final height explained by the variation in birth length or height at later ages, or both (table 1). The older the age included, the higher was the proportion explained. Of the variation in final height, 30% for boys and 46% for girls were explained by the variation in the heights of both parents. These proportions improved to 53% and 57%, respectively, after addition of the children's own heights at the age of 1·0 year.

We developed two equations to improve the accuracy of evaluation of height (table 2). The first includes parental heights and defines parent specific mean standard deviation scores. The second also includes the child's height at the age of 1·0 year and defines the expected standard deviation score. We compared these with a simple, easy to memorise equation according to which the parent specific mean standard deviation score is half the mean standard deviation score of the parents.[5] On average the difference between the final height measured and that expected from these equations was small (table 3). In practice, the two new equations are converted to nomograms (fig 2).

The mean (SD) age at menarche of the mother—14·1 (1·6) years—correlated positively with the daughter's age at menarche—12·9 (1·2) years—(n=519, r=0·28, $p<0·001$)—her age at 'take off'—8·5 (1·1) years, and her age at peak height velocity—11·4 (1·1) years (n=179, r=0·16, $p<0·05$, in both cases). The ages at 'take off' and at peak height velocity were lower for the children whose fathers had reached their final height early (table 4).

Discussion

About 20% of the variation in height at any

Table 4 *Father and child relationships in the timing of maturation*

	Timing of father's puberty		
	Early	*Average*	*Late*
Boys:	(n=27)	(n=178)	(n=47)
Age at take off	9·4 (1·3)	9·7 (1·4)	9·8 (1·3)
Age at peak height velocity	12·8 (1·5)**	13·6 (1·1)	13·7 (1·0)
Girls:	(n=16)	(n=124)	(n=31)
Age at take off	8·1 (0·9)	8·7 (1·3)	8·3 (0·8)
Age at peak height velocity	10·9 (0·9)	11·5 (1·1)	11·3 (1·4)

Values are mean (SD) years. **$p<0·01$.

prepubertal age, and 30–46% of the variation in final height, was explained by the variation in parental heights. The inclusion of the children's own heights at the age of 1·0 year increased the proportion explained to 50%. Tanner *et al* reported that the percentage of adult variance that could be accounted for by the data about heights at the ages of 0, 1, 2, 3, 4, and 5 years was 67% in males and 64% in females.[6] He concluded that the principal determinant of the size of a newborn baby is the prenatal environment, which depends on hereditary characteristics of the mother, the influence of the inherent growth characteristics of the child appears only later.[6] He considered that adult height can be predicted equally well at the ages of 3, 4, and 5 years.

Tanner *et al* published a chart for calculating height standards that allowed for the parents' heights at the ages of 2 to 9 years.[1] In this chart the standards are in the form of a regression line (child's height against midparental height) with standard deviations calculated from the average sex specific standard deviation of the general population multiplied by 0·87.

Tanner also described a 'target height method' in which a child's predicted final height is compared with a target range of height that can be expected from the heights of the parents.[7] The centre of the target range is midparental height plus 6·5 cm for boys and midparental height minus 6·5 cm for girls (6·5 cm being half the mean sex difference in adult height). The target range is two standard deviations below and above this figure, and the standard deviation equals the sex specific standard deviation of the general population multiplied by 0·77.[7] Tanner's two methods differ essentially from each other, the first implies that the mean height standard deviation score of children is half the mean score of the parents (our simple equation 2), but the second method takes the straight mean standard deviation score of the parents as being the mean score of the children.

Our data may be criticised for lack of accuracy because the measurements were made within the primary health care system, and especially because the timing of parental maturation was based on recollection. Obtaining accurate longitudinal data would require almost two decades and—more importantly—it is with the same kind of less than perfect data that child health surveillance will always have to deal when monitoring children's growth. The Finnish child health surveillance system has a long tradition of measuring every child annually. We believe that our data are the best possible in the absence of longitudinal measurements by anthropometrists.

We calculated parent specific mean height standard deviation scores and the child specific expected standard deviation score. The latter can be used for detecting an acquired growth disorder in children whose growth during the first year of life is believed to be normal. We chose the age of 1 year instead of the ages of 2 or 3 years, though the latter correlated better with the final height. We did this because we wanted to minimise the risk that an acquired non-diagnosed disease might already have influenced the height used in the prediction, and also to maximise the age range at which the prediction could be used. These equations may be used easily as nomograms. The standard deviations of the differences between the measured final heights and those expected from these two equations were 4·3 to 5·3 cm for boys and 3·7 to 4·9 cm for girls, which is clearly less than the standard deviations of final height in the general population (6·0 cm for men and 5·4 cm for women). Interestingly, the simple calculation of parent specific height standard deviation score by taking mean parental height (cm), subtracting 170 and dividing by 10, proved to be as accurate as the more complex equation developed from our present data. The simple equation has been derived[5] from the complex chart of Tanner *et al.*[1] These equations are not proposed to predict final height, but to improve detection of abnormal growth in child health surveillance. A child's height standard deviation score at different ages should be compared with the expected parent specific and child specific score. We have recommended that if a child's actual standard deviation score differs by more than 2·0 from the parent specific mean score, or more than 1·5 from the expected score, this should arouse suspicion of a growth disorder. We have incorporated the parent and child specific height standard deviation scores into a 'growth screen'—a set of rules defining (for child health surveillance) which kinds of growth are acceptable and which kinds call for the attention of a specialist. This screen also includes limits for changes in height standard deviation scores.[8 9]

The ages at 'take off' and at peak height velocity were similar in our series to these of Preece and Heinrich, who obtained them using mathematical models of individual growth curves.[2] They were about 1 year lower than in the study of Largo *et al*, in which velocity curves were analysed using smoothing spline functions, as in our study.[10]

The ages at menarche of the daughters and mothers were positively related in our study, as in another.[11] We found similar correlations between the age at menarche of the mothers and the ages at 'take off' and at the peak height velocity of the daughters. The rough estimate of timing of maturation of the fathers was also positively correlated with that of the children, especially the sons. We have completed our growth screen with a puberty screen in which the screening limits are defined by 95% confidence intervals of the population for pubertal acceleration of growth, and the ages at start of growth of breasts, male genitals, and pubic hair. We consider that if the timing of maturation of one or other parent has deviated from the population mean by at least 1 year, we accept that the timing of maturation of the child may exceed the screening limits by at most 1 year in the same direction. By this we mean that there is no need for diagnostic evaluation. Young people who differ clearly in timing of maturation from their peers, however, need information about why they are different, and what will be the course of their development.

In conclusion, we have developed rules for application of information of the parents' heights and maturation history to the evaluation of the growth and maturation of children. Our rules lack scientific accuracy, but we prefer using them to having no rules at all to offer for child health surveillance.

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References

[1] Tanner JM, Goldstein H, Whitehouse RH. Standards for children's height at ages 2–9 years allowing for height of parents. *Arch Dis Child* 1970;**45**:755–62.

[2] Preece MA, Heinrich I. Mathematical modelling of individual growth curves. *Br Med Bull* 1981;**37**:247–52.

[3] De Boor C. *A practical guide to splines. Applied mathematical sciences.* New York: Springer Verlag, 1974.

[4] Dixon WJ. *BMDP statistical software.* Berkeley: University California Press, 1981.

[5] Perheentupa J. Lapsuuden ja nuoruuden endokrinologiaa. In: Lamberg B-A, Nikkilä E, Pelkonen R, eds. *Kliininen endokrinologia.* 2nd ed. Helsinki: Duodecim, 1984:514–94.

[6] Tanner JM, Healy MJR, Lockhart RD, Mackëzie JD, Whitehouse RH. Aberdeen growth study. 1. The prediction of adult body measurements from measurements taken each year from birth to 5 years. *Arch Dis Child* 1956;**31**:372–81.

[7] Tanner JM. Use and abuse of growth standards. In: Falkner F, Tanner JM, eds. *Human growth.* Vol III. 2nd ed. New York: Plenum Press, 1986.

[8] Sorva R, Tolppanen E-M, Lankinen S, Perheentupa J. Variation of postinfantile growth in height and weight. An analysis of 2000 children. *Acta Paediatr Scand* (in press).

[9] Sorva R, Tolppanen E-M, Perheentupa J. Variation of growth in length and weight in infancy. *Acta Paediatr Scand* (in press).

[10] Largo RH, Gasser TH, Prader A, Stuetzle W, Huber PJ. Analysis of the adolescent growth spurt using smoothing spline functions. *Ann Hum Biol* 1978;**5**:421–34.

[11] Kantero R-L, Widholm O. A statistical analysis of the menstrual patterns of 8000 Finnish girls and their mothers. *Acta Obstet Gynecol Scand* 1971;**30**:1–35.

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Screening infants for hearing loss

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SUMMARY A computerised child health register was used to study the coverage, referral rate, and false positive rate of the eight month hearing distraction test in a cohort of 1990 births to residents of one district during an eight month period. Coverage by the age of 9 months was under 60% and varied with ethnic group and immunisation record. The true problem rate among those referred was 48%. None of the three children in the cohort who had a sensorineural hearing loss was picked up by screening, although it did identify children with conductive loss. The findings question the value of the distraction test as currently used, and underline the usefulness of computerisation, even if limited to child registration, in the evaluation of screening tests.

Without prompt preventive action, deafness in the early years of life can hinder the development of communication. In order to identify affected children as early as possible the Department of Health and Social Security (DHSS) recommended that all infants be screened at about the age of 8 months, using the 'distraction' test, and that this should be done by health visitors because of their role in the assessment of children and in the counselling of parents.[1] Most district health authorities have now implemented early screening for hearing loss for all children, followed by testing of those who are 'screened out' by specially trained clinical medical officers and—where appropriate—audiologists. This programme is complex, and its effectiveness is reduced by the poor sensitivity and specificity of the screening test, and problems of coverage and follow up in areas where the population is mobile.[2-8] Because of this complexity few districts have been able to evaluate their own programmes.[9]

This paper evaluates the process and effectiveness of the programme in one district health authority in an inner city. The investigation was planned as part of a larger study supported by the DHSS of the efficiency, effectiveness, and cost of child health services.

Subjects and methods

RECOMMENDED PROGRAMME OF HEARING SCREENING IN THE STUDY DISTRICT

The health visitors arrange to see all the children when they have reached the age of 8 months, preferably in a clinic, and at a time when a trained colleague is available to assist. The distraction hearing test is carried out according to the recommended method, and if the response is not satisfactory the child is given an appointment for a repeat test, unless the health visitor is so concerned that she refers the child immediately for an audiological assessment. In some cases children are not referred unless a third or even fourth test is also unsatisfactory. Failure of a test may be the result of a respiratory infection, or failure to cooperate, or because of a genuine hearing defect.

A child referred because of an unsatisfactory screen is normally given an appointment for a secondary audiology clinic held by a senior clinical medical officer and a speech therapist. The purpose of these is to separate false positives from those screened out and to refer for tertiary audiology those children with confirmed serious hearing problems. Children with a mild and apparently temporary loss are counselled or referred to the general practitioner or the hospital as appropriate.

The purpose of the tertiary audiology clinic is primarily to assess those children who are difficult to test and those with sensorineural hearing loss, and where appropriate to prescribe hearing aids.

In addition to the screening services offered by the community child health services, general practitioners may also refer children directly for an audiological assessment. They are requested to make all referrals to the secondary and tertiary audiology clinics through the community child health services to ensure that children are not given two appointments. Consultant paediatricians may also refer children directly to the secondary or tertiary audiology clinics. Neither clinic has 'open access' to parents who are concerned about their child's hearing.

The DHSS guidelines recommend that infants should be screened when they are as near the age of 8 months as possible, and for this reason the present study focused on the tests carried out before 10 months.

METHOD

Sample studied and data collection

No data were readily available, but the health visitors had been using a standard form completed by hand to record the date of the test and the child's response to each stimulus during his or her first and, if necessary, second test. This form was redesigned to make it computer compatible, and to include additional information such as the action taken as a result of the screen. It was then used as the basis of a prospective study of a cohort of 1990 children born between 7 August 1985 and 31 March 1986.

The computerised child health register was used to produce preprinted labels for every eligible child in the birth cohort resident in the district in April 1986. The information printed on each label included the child's 'soundex' code, which is a unique identifier generated by the computer and made up from a code derived from the child's last name, date of birth, sex, and a suffix code and check digit. Also printed on each label was the child's name and address and codes identifying the child's health visitor, the child health clinic or health centre, and the name of the general practitioner. Each label was attached to one of the redesigned forms and delivered to the child's health visitor for completion. Forms without labels were provided for children born between 7 August 1985 and 31 March 1986 who attended a clinic for a hearing test but for whom no prelabelled form was available (perhaps because he or she had recently moved into the district). For these a section was added to be completed with the child's name, address, date of birth, and sex. Each form had a carbonned second sheet that was identified with either the soundex code or the name and address if no label was supplied. Health visitors were asked to keep the top copy for their records and return the carbon copy to the researchers.

The completed forms were collected for those children in the cohort tested up to the end of November 1986. Health visitors were also asked to return the forms of children who were not screened because they had not attended, moved, or died.

Matching of data to child register

The completed forms were then matched by their soundex code to their records on the child health register. For those children for whom no preprinted label had been supplied, the section completed by the health visitor giving the child's name, address, date of birth, and sex made it possible to match data with that kept on microfiche by the district's community child health services and to select a soundex code from this. The number of children in the cohort for whom no screening results were received could then be ascertained.

Referral data

The district's community child health service keeps a card index of each child for whom a referral appointment has been made giving the name, address, and audiology clinic concerned. This allowed us to abstract from clinic notes information on the diagnosis or action taken as a result of the audiological assessment. Children who were to have their test repeated again after their second test were also followed up by asking the health visitors what action was subsequently taken.

Children tested elsewhere

The soundex codes of 10 children who had been tested or assessed elsewhere were also noted and their names and addresses obtained in the same way. The health visitor of three of these children indicated that they had passed their test, but the remainder were followed up to see if an appointment had been made by the community child health services for a secondary or tertiary audiology clinic. Three children of low birth weight and two others were referred directly for an auditory evoked brain stem test.

Validation exercise on those reported not screened

In some cases failure to screen may have been accounted for by the fact that the children would only just have reached the age of 9 months by the last month of data collection, or that forms for other eligible children may not have been returned. To investigate those non-responders a random approximate 10% sample was taken, after excluding 10 children who were known to have moved away, 27 who had failed to attend, and those who had weighed less than 1500 g at birth (who are usually referred directly for tests). The relevant health visitors were then asked whether the remaining children in this sample had actually been screened and if so the date of the test, or, the reason why it had not been carried out.

Results

COVERAGE

Of the 1990 children in the cohort, 1109 forms were

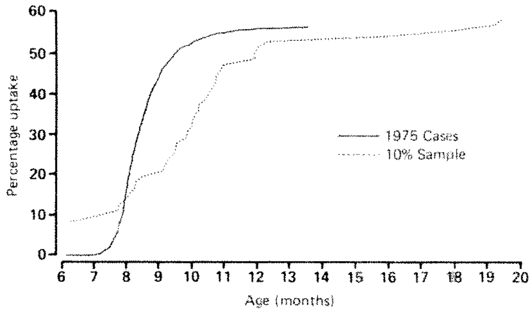

Fig 1 *Cumulative percentage uptake rate for total 1975 cases eligible, and for the 10% sample.*

returned indicating that the child had been screened. As stated earlier a further 15 children had been screened elsewhere or it was found they had already had an audiological assessment. No screening results were received for the remaining 866 children. This implied an overall uptake rate in the study cohort of 56%, excluding the 15 children screened or assessed elsewhere. The mean age at screening for these 1109 children was 8·6 months and the cumulative percentage coverage rate, ascending steeply between 8–9 months, is shown in fig 1. Data on the age at screening was missing for only one child.

The approximate 10% sample of those for whom no screening results were received comprised 77 cases. Thirty one (40%) had failed to attend for their screening test. The most common reason given (accounting for 15 of the 31) was that the health visitor was unable to persuade the parent to attend the clinic or to carry out the test in the child's home. Another 14 had either moved or were part of the district's substantial Bangladeshi community (who often return to their home country for several months at a time). Two children had been incorrectly entered on the regional child health register and were not in the cohort.

Forty three children (56%) had actually been screened but no form returned. A further child was untraceable, and two were tested elsewhere. The mean age at screening was available for 37, and was 10·7 months, significantly higher than for the 1109 children for whom results were received ($p \leq 0\cdot0001$). This is reflected in the difference in the cumulative percentage uptake rate shown in fig 1.

The results from the original completed forms correctly reflected the coverage rate up to 9 months of age, though using data from the 10% sample an estimated 72% were screened up to the age of 10 months, and 80% up to the time of analysis (fig 1). Many were tested after the age for which the test is designed, and after a time at which they would have been screened again by clinical medical officers. The results that follow apply only to the 1109 cases said to have been screened in the original cohort.

Table 1 *Percentage coverage and mean age at screening, by sex, ethnic group, mothers' age, and birth weight*

Characteristics	*No of eligible children*	*Total No tested*	*Percentage uptake*	*Mean age at screening (months)*
Sex:				
Male	1014	553	54·5	8·60
Female	961	446	46·4	8·56*
Ethnic group:				
Asian	743	413	55·6	8·62
White	746	457	61·3 †	8·54
Other	175	109	62·3	8·55*
Not known	311	130	41·8	8·62
Mothers' age:				
<20	220	133	60·5	8·53
20–24	587	329	56·0	8·62
25–29	512	311	60·7	8·60
30–34	281	148	52·7	8·50
≥35	182	114	62·6	8·41*
Not known	193	74	38·3	8·79
Birth weight (g):				
<1500	9	5	55·6	9·86
1500–2499	139	80	57·6	8·60
≥2500	1655	960	58·0	8·55*
Not known	172	64	37·2	8·84

*Data on age at screening missing for one case; †$p<0\cdot05$, χ^2 (2 DF) 5·91.

FACTORS AFFECTING COVERAGE

The linkage with the child health register meant that the percentage uptake rate and mean age at screening could be analysed by the children's sex and ethnic group, maternal age at birth, and birth weight (table 1), excluding the 15 children tested elsewhere.

For boys the coverage was lower and mean age at screening higher than for girls but not significantly. The coverage for Asian children was significantly lower than that for other children and the mean age at screening higher but not significantly. There was no obvious pattern for coverage and mean age at screening by maternal age. Coverage for infants who had weighed less than 1500 g at birth was lower and the mean age at screening higher (but not significantly) than for the remaining babies, but it is known that these are referred directly.

Use of the child health register data also enabled a comparison to be made between rates of uptake of the first 'triple' immunisation for children who had and who had not been screened. The results (table 2) show that the immunisation uptake rate of those screened for hearing loss is significantly greater than of those who had not been screened.

The coverage of the 317 children with health visitors attached to their general practice is higher, but not significantly so (60%), than that of those whose health visitors were not attached to a practice (56%) (table 3), and very much lower for those for whom data were not available.

Table 2 *Comparison of number of children immunised and number of children screened*

	Total No	*No (%) who had received first dose of triple vaccine*		*p Value*
Screened	1109	1049	(94·6)	<0·001*
Not screened	866	682	(78·8)	
Total	1975	1731	(87·6)	

*z=10·63

Table 3 *Comparison of screening uptake rate between children whose health visitors were attached to a general practice and those who were not*

	Total No	*No (%) screened*
Health visitor attached to a general practice	317	189 (59·6)
Health visitor not attached to a general practice	1644	917 (55·8)
Not known	14	3 (21·4)
Total	1975	1109 (56·2)

ACTION TAKEN AS THE RESULT OF SCREENING

Fig 2 illustrates the complexity of the action taken as a result of screening. Of the 1109 children screened 888 (80%) 'passed' their screen and 207 (19%) were asked to return for a repeat test. Six of the 207 were also referred to their general practitioners, and 20 to a clinical medical officer. Three other children were referred to a clinical medical officer, and eight children (1%) were directly referred for a full audiological assessment. One other child, a preterm infant, was referred directly to the district's child development ('disability') team.

Table 4 gives the outcome of the first test by sex, ethnic group, maternal age at birth, and birth weight. The proportion of infants thought to have a 'problem' as a result of their screen (those who were to have their tests repeated or who were referred for further investigation) is significantly greater for boys and for Asians, but maternal age at birth was not associated with outcome. The analysis by birth weight suggests that a higher proportion of infants of low birth weight thought to have a 'problem' as a result of their first screening test is not statistically robust, but those at highest risk tend to be directly referred.

Of the 207 children recalled for a second test 120 (58%) 'passed', 14 (7%) did not attend, 18 (9%) were to have their tests repeated, 16 (8%) were referred to their clinical medical officer, and 39 (19%) were referred for a full audiological assessment. Of the three referred to their clinical medical officer, one did not attend a second time and the other two were referred for a full audiological assessment.

Of the 18 children recalled for a third test, 12 (67%) passed, four (22%) did not attend, one (6%) was recalled for a fourth test (which was satisfactory), and one (6%) was referred for a full audiological assessment. Of the 16 referred to their clinical medical officer after their second test, 10 (63%) passed, one (6%) did not attend, and five (13%) were referred for a full audiological assessment.

Thus of the 1109 children screened 55 (5%) were eventually referred for audiological assessment as a result of the screening process, one child was referred by his general practioner because of wax in his ears, and another was referred back to the district's child development team. The last child was subsequently referred for full audiological assessment.

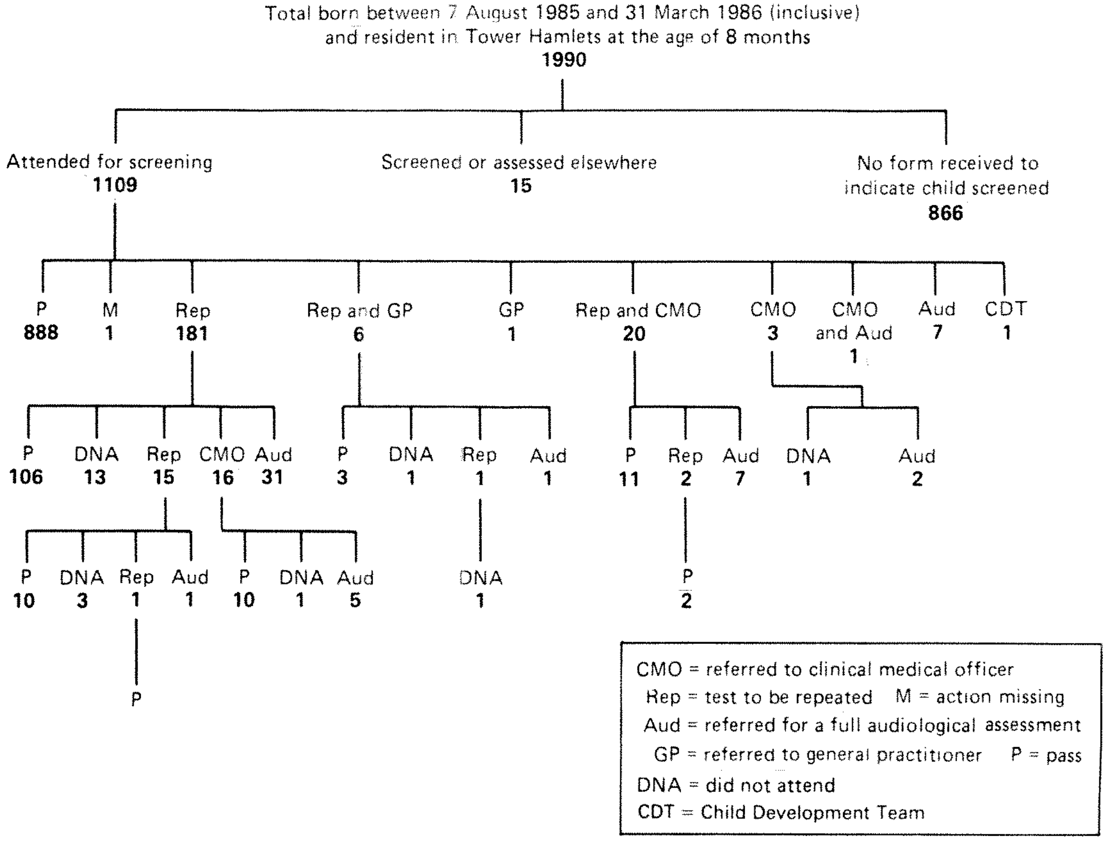
Total born between 7 August 1985 and 31 March 1986 (inclusive)
and resident in Tower Hamlets at the age of 8 months
1990
Attended for screening
1109
Screened or assessed elsewhere
15
No form received to indicate child screened
866
P
888
M
1
Rep
181
Rep and GP
6
GP
1
Rep and CMO
20
CMO
3
CMO and Aud
1
Aud
7
CDT
1
P
106
DNA
13
Rep
15
CMO
16
Aud
31
P
3
DNA
1
Rep
1
Aud
1
P
11
Rep
2
Aud
7
DNA
1
Aud
2
P
10
DNA
3
Rep
1
Aud
1
P
10
DNA
1
Aud
5
DNA
1
P
2
P
CMO = referred to clinical medical officer
Rep = test to be repeated
M = action missing
Aud = referred for a full audiological assessment
GP = referred to general practitioner
P = pass
DNA = did not attend
CDT = Child Development Team

Fig 2 *Flow diagram showing action taken as a result of screening.*

CHILDREN REFERRED FOR AUDIOLOGY AS A RESULT OF SCREENING

Fig 3 shows that no appointment was made for two of the 55 children in the main study referred for a full audiological assessment. Six children (11%) did not attend and were discharged back to their health visitor. Of the remainder, 18 children (38%) were diagnosed as having mild conductive hearing loss, of whom six were subsequently referred to a hospital ear, nose, and throat (ENT) department. A further four children also had problems related to hearing, namely wax in the ears or flat tympanic membranes. No further action was taken for two of these: one was referred back to the child development team and one referred to the general practitioner.

The baby already under the care of the district's child development team who had failed its first test was also referred to the secondary audiology clinic, and subsequently referred to a hospital ENT department for a mild conductive hearing loss.

PREVALENCE OF HEARING PROBLEMS IN CHILDREN WHO WERE FOUND TO HAVE A PROBLEM ON SCREENING

In summary 48 of the children were seen at an audiology clinic, of whom 25 (52%) were discharged without a problem being found. The remaining 23 (48%) had confirmed problems, none of which were sensorineural. Twenty children (1·8% of the 1109 screened) were confirmed to have some degree of conductive deafness picked up by the screening, including two who had been screened elsewhere.

It is of some interest that of the 43 children in the 10% sample who were screened but whose forms were not received four (9·3%) were found to have a conductive hearing loss, a considerably higher proportion than in the main study.

PREVALENCE OF SENSORINEURAL DEAFNESS IN THE COHORT

There were three children in the birth cohort who, by the end of the study, were known to have

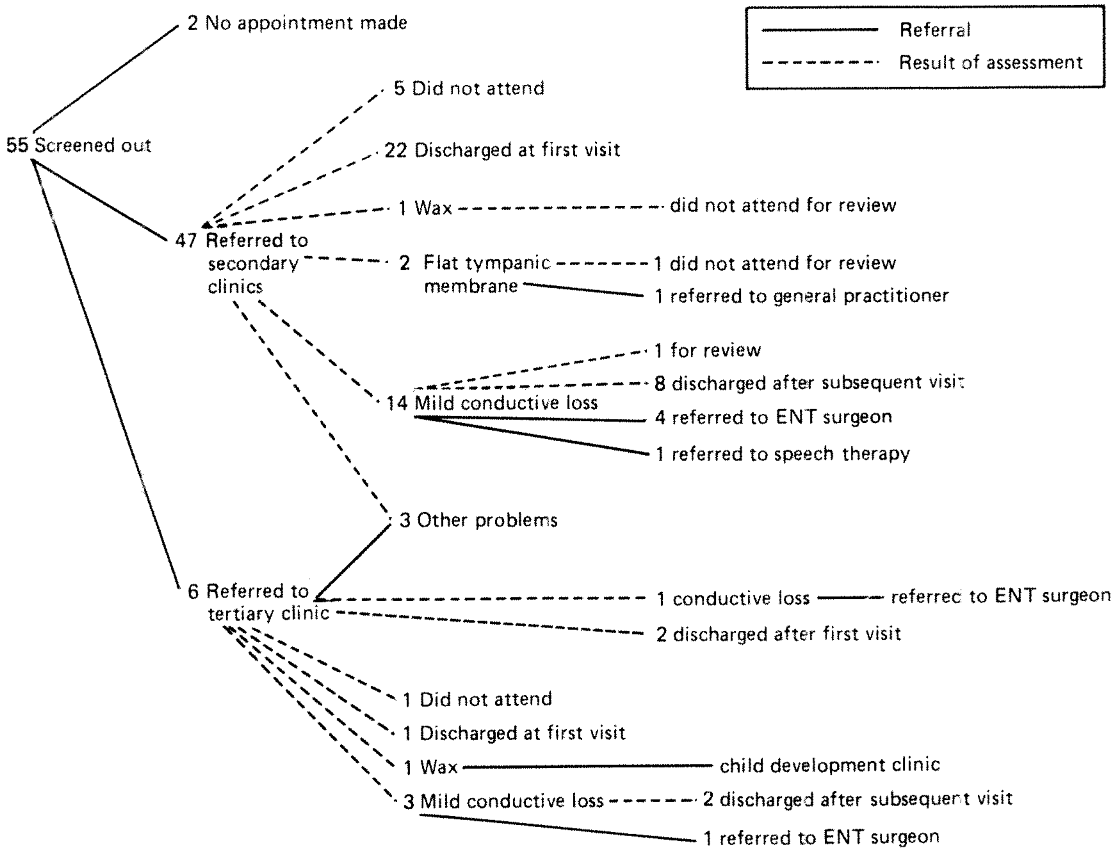
Referral
Result of assessment
55 Screened out
2 No appointment made
47 Referred to secondary clinics
5 Did not attend
22 Discharged at first visit
1 Wax
did not attend for review
2 Flat tympanic membrane
1 did not attend for review
1 referred to general practitioner
14 Mild conductive loss
1 for review
8 discharged after subsequent visit
4 referred to ENT surgeon
1 referred to speech therapy
3 Other problems
6 Referred to tertiary clinic
1 conductive loss
referred to ENT surgeon
2 discharged after first visit
1 Did not attend
1 Discharged at first visit
1 Wax
child development clinic
3 Mild conductive loss
2 discharged after subsequent visit
1 referred to ENT surgeon

Fig 3 *Intermediate outcome of children referred for full audiological assessment.*

sensorineural hearing loss. One child, diagnosed at 18 months as having mild sensorineural hearing loss, had been screened at the age of 8 months and no defect was reported. The deafness was detected at the tertiary audiology clinic when he attended with his brother who had failed his school screen, accompanied by their deaf father; it seems likely that the hearing loss had been present at 8 months.

Of the five children referred directly (see methods) two were found to have mild sensorineural hearing loss diagnosed by an auditory evoked brain stem test. One of the children was also blind and the other had cerebral palsy.

TIME BETWEEN REFERRAL AND THE FIRST APPOINTMENT

Urgent cases were given priority, but the mean overall time between a child's test and a subsequent audiology appointment was 4·7 months. For the 22 children found to have a problem the mean waiting time was 4·0 months (median 5·3 months), lower (but not significantly so) than the mean of 5·1 months for the 31 remaining children who were seen at a secondary audiology clinic.

The maximum waiting time after screening was 15·2 months, but this occurred when the health visitor concerned was on sick leave for eight months. If the latter child is excluded there was a maximum waiting time of 9·8 months (mean 4·5, and median 5·3).

Discussion

Although the distraction screening for hearing loss was originally introduced to identify (so permitting special care for) infants with sensorineural hearing loss, the current thinking is that it is also important to detect children with conductive hearing loss. It is thought that these children benefit from the counselling given to their parents, as well as the treatment

Table 4 *Results of the first test by sex, ethnic group, mothers' age, and birth weight*

Characteristics	*No tested*	*Percentage with 'problem'*	*p Value*
Sex:			
Male	553*	22·4	<0·05†
Female	556	17·3	
Ethnic group:			
Asian	413	25·9	<0·05‡
White	457	16·6	
Other	109	16·5	
Not known	130*	14·6	
Mothers' age:			
<20	133	15·8	
20–24	329	21·9	
25–29	311	19·0	
30–34	148	20·9	
35+	114	22·8	
Not known	74	14·9	
Birth weight (g):			
<1500	5	60·0	
1500–2499	80	26·3	
≥2500	960	19·4	
Not known	64	15·6	

*Data on action taken missing for one case; †z 2·17; ‡χ^2 (2 DF) 12·66.

they receive, including speech therapy. Our results have shown that infants with conductive hearing loss were being picked up by the screening programme, but that children with sensorineural hearing loss were being detected by other means. If the primary aim of the screen is becoming the detecting of conductive hearing loss it should perhaps be carried out later, as Haggard and Gannon have pointed out, as the optimum age for detecting conductive hearing losses is over 8 months.[10]

It would have been of interest to have collected data on the number of children with conductive hearing loss apparently missed by the screen, but this would have required a second and more skilled screening of the whole group. Moreover, in view of the constantly changing nature of such a loss one would not expect a high degree of repeatability.

In the present study the screening coverage was 56% by the age of 9 months, increasing to about 80% by relaxing the screening age cut off point. There was a significant deficit in coverage of Asians, and a small but not significant deficit of coverage of boys. Reports from other districts have described overall coverage rates for the routine infant screen of between 50% to 90% but we could find no more detailed data comparable with ours.[3–5 8]

The analysis of the Nottingham system included an investigation into the variation of the failure and referral rates between groups of health visitors based at different health centres.[10 11] The variation found raised the question of whether the test as carried out is an objective and repeatable procedure. The fact that a decision to refer a child for a full audiological assessment may be made after taking account of factors other than the result of the test is also reflected in the eight different pathways that we have described by which children reached an audiology clinic, many children seeing the clinical medical officer or their general practitioner, and some having their test repeated more than twice before a final decision was made to refer. As Haggard and Gannon stated, the subjective factors within the screening procedure may well be efficient but they are unspecified.[10] The testing procedure needs further examination.

The referral rate for the present study was 5% and the percentage found to have a problem at an audiology clinic was 48. Comparable studies of the Nottingham system showed an original referral rate of 2·8% and a true problem rate of 59·3% in 1981, which had increased to 4·1% and 78·6%, respectively, by 1984, reflecting the improvements introduced as a result of the working party set up to look into the programme.[10 11] These included the offer of open access for full audiological assessment, a revised training system and refresher courses, plus the introduction of a 'clue list', to alert parents to the possibility of deafness in their babies, used to supplement the community screening.[4 5 7 10–13]

In comparison with the latter figures, the study district had a slightly lower threshold for referral and a lower proportion of true positives, and there is therefore scope for improvement. Trials of the effect of introducing the 'clue list' are under discussion.

The computerisation of preschool health programmes will make it easier to introduce trials of the long term effectiveness of different types of screening, including the consequences of early treatment of conductive deafness and ways of reducing non-attendance. There is an urgent need for such studies to justify the continuation of the 8 month distraction screen, which is a time consuming programme, the resources for which may be put to better use.

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References

[1] DHSS Advisory Committee on Services for Hearing Impaired People. *Final report of the subcommittee appointed to consider services for hearing impaired children*. London: HMSO, 1981.

[2] Health Visitors' Association. Hearing screening survey no 2. *Health Visitor* 1981;**56:**191.

[3] Bellman S. Hearing screening in infancy. *Arch Dis Child* 1986;**61:**637–8.

[4] McCormick B. Hearing screening by health visitors: a critical appraisal of the distraction test. *Health Visitor* 1983;**56:**449–51.

[5] McCormick B. Screening for hearing impairment in the first year of life. *Midwife, Health Visitor and Community Nurse* 1986;**22:**199–202.

[6] Robinson K. The scandal of late diagnosis of deafness in children. *Health Visitor* 1983;**56:**452–3.

[7] Latham AD, Haggard MP. A pilot study to detect hearing impairment in the young. *Midwife, Health Visitor and Community Nurse* 1980;**16:**370–4.

[8] Boothman R. Identifying the deaf child: how accurate are simple tests? *Modern Medicine* 1979;**24:**26–30.

[9] Stewart-Brown S, Haslum MN. Screening for hearing loss in childhood: a study of national practice. *Br Med J* 1987;**294:** 1386–8.

[10] Haggard MP, Gannon MM. *Analysis from service records of screening systems for hearing impairment in pre-school children*. Internal Report Series A, No 3. Nottingham: Medical Research Council Institute of Hearing Research, 1985.

[11] Haggard MP. Monitoring the efficiency of hearing screens for the first year of life. *Audiology in Practice* 1986;**3:**3–5.

[12] McCormick B, Wood SA, Cope Y, *et al*. Analysis of records from an open-access audiology service. *Br J Audiol* 1984;**18:** 127–32.

[13] Hitchings V, Haggard MP. Incorporation of parental suspicions in screening infants' hearing. *Br J Audiol* 1983;**18:**71–5.

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Complications of diazoxide treatment in persistent neonatal hyperinsulinism

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SUMMARY Seven infants with persistent neonatal hyperinsulinism were treated in Dhahran Health Centre from 1983 to 1986. The insulin:glucose ratio (serum insulin concentration pmol/l) divided by the blood glucose concentration (mmol/l) ranged from 12 to 636, mean (SD) 177 (201). To control hypoglycaemia, diazoxide (12–24 mg/kg/day) was given in a continuous intravenous glucose infusion (12–22 mg/kg/min) on 11 separate occasions, four infants twice each and three infants once each. An increase of more than one standard deviation in the heart and respiratory rates, together with other symptoms of heart failure, was considered to be evidence of diazoxide toxicity. Cardiorespiratory failure (toxicity) occurred on eight of the 11 occasions (73%) in seven infants. The average daily fluid intake, weight change, respiratory rate and heart rate before treatment were similar whether or not the infant developed toxicity. A diazoxide toxicity index was obtained by multiplying the dose of diazoxide by the insulin:glucose ratio to relate the diazoxide dose to the severity of the disease. In all instances when the toxicity index was more than 1533 (mean (SD) 3732 (2741)) cardiac toxicity developed. In contrast, infants with a toxicity index of less than 675 (mean (SD) 364 (270)), had no symptoms of toxicity. Symptoms were significantly related to the severity of the disease and the diazoxide dose. It is possible to use the toxicity index to predict the risk of toxicity and to calculate a safe dose of diazoxide in infants with persistent neonatal hyperinsulinism.

Persistent neonatal hyperinsulinism is an uncommon disease characterised by hyperplasia of the β cells of the pancreas and persistent inappropriate hyperinsulinaemia and hypoglycaemia.[1–3] Medical treatment of the associated hypoglycaemia is often difficult despite adequate glucose intake and various drug regimens. Diazoxide, a non-diuretic benzothiadizine, has been used in various doses to achieve euglycaemia.[3–5] Cardiorespiratory failure has been reported,[4–6] the cause of which is not clear. We report the complications encountered during diazoxide treatment of seven patients with persistent neonatal hyperinsulinism, and discuss the possible causes for cardiorespiratory failure.

Subjects and methods

During the years 1983–6 seven infants with persistent neonatal hyperinsulinism were admitted to Dhahran Health Centre in the eastern province of Saudi Arabia, where a high incidence of persistent neonatal hyperinsulinism (one in 2675 live births) has been reported.[7]

There were three boys and four girls, all born at full term. Birth weights ranged from 3640 to 5020 g (mean (SD) 4054 (448) g); three were large for gestational age. All parents had consanguinous marriages. Details of the clinical characteristics and presentation have been reported elsewhere.[7] All seven infants had persistent refractory hypoglycaemia (blood glucose concentration <2·22 mmol/l with a glucose intake of >12 mg/kg/minute) in association with inappropriate hyperinsulinaemia.

Histopathological studies of the pancreas from all seven patients showed generalised hyperplasia of the β cells. Medical management was attempted for two to four weeks before pancreatectomy; this included intravenous glucose infusion from 12 to 22 mg/kg/min, glucagon, corticosteroids, adrenaline, epinephrine, chlorothiazide, and diazoxide in various doses and combinations. Full details of the medical management has been reported elsewhere.[7]

Diazoxide was given in 11 instances (four infants twice each, and three infants once each) in doses ranging from 12 to 24 mg/kg/day for varying periods. All seven patients were given a trial of diazoxide before pancreatectomy was carried out. In addition, cases 1, 2, and 3 were given diazoxide after partial pancreatectomy for hypoglycaemia, and case 4 received diazoxide in two different trials before pancreatectomy.

To determine the side effects of diazoxide, documentation of signs and symptoms of heart failure were looked for retrospectively. The average respiratory rate and heart rate before and during treatment with diazoxide were calculated from the two hourly nursing observations. Weight was measured daily, and average weight gain (g/kg/day) was calculated daily; the average daily fluid intake during treatment was also calculated. Chest radiographs were evaluated and the cardiothoracic ratio was determined. The electrocardiograms and echocardiograms were reviewed by an independent cardiologist.

The degree of inappropriate hyperinsulinaemia was expressed by the ratio of serum insulin (pmol/l) to blood glucose concentration (mmol/l). To compare the effects of diazoxide in relation to the severity of the disease as judged by the insulin: glucose ratio, a toxicity index was calculated by multiplying the insulin:glucose ratio by the diazoxide dose (mg/kg/day).

Diazoxide toxicity was diagnosed if the following criteria were present: the heart rate and respiratory rate had increased by more than one standard deviation from the mean rate before treatment, and the infant developed cardiomegaly, heart mumur, or hepatomegaly. Student's *t* test and Fisher's exact test were used for assessing the significance of differences as appropriate.

Results

Three boys and four girls met the criteria of persistent neonatal hyperinsulinism during the study period (table 1). In three of 11 instances of diazoxide treatment, no significant changes occurred in the cardiorespiratory state, but in the remaining eight signs of cardiorespiratory failure developed (table 2).

The serum insulin concentrations ranged between 29 and 843 pmol/l, and the insulin:glucose ratio ranged between 12 and 636 (table 1). Blood glucose tended to be lower and serum insulin higher in the infants who developed symptoms of toxicity compared with those who did not ($p<0·1$ and $p<0·025$, respectively). Symptoms of cardiorespiratory failure occurred whenever the insulin:glucose ratio rose above 63, and in none of the infants with a glucose insulin/ratio of less than 33. Even case 6 is excluded (his insulin:glucose ratio was more than two standard deviations above the mean) there was a significant difference in insulin:glucose ratio between those who developed complications and those who did not ($p<0·02$).

The toxicity index range between 191 and 9543. It was significantly higher in infants who were toxic than in those who were not ($p<0·011$). All patients with toxicity indexes of 675 and below had no symptoms of toxicity. In contrast, all patients with

Table 1 *Insulin and glucose concentrations and diazoxide toxicity index*

Case No	*Insulin (pmol/l)*	*Glucose (mmol/l)*	*Insulin:glucose ratio*	*Diazoxide (mg/kg/day)*	*Toxicity index*
Toxic infants:					
1	307	1·056	291	18	5238
1*	121	1·056	115	18	2071
2	400	2·333	171	12	2057
3	107	1·315	83	24	1995
4	89	1·417	64	24	1533
5	111	0·972	107	23	2455
6	843	1·361	636	15	9543
7	386	1·167	331	15	4959
Mean (SD)	296 (257)	1·334 (0·434)	225 (193)	18·6 (4·6)	3732 (2741)
Infants who were not toxic:					
2*	29	1·889	15	15	227
3*	50	1·556	32	21	675
4	29	2·389	12	16	191
Mean (SD)	36 (12)	1·944 (0·419)	20 (11)	17 (3·2)	364 (270)
p Value†	0·025	0·123	0·02	0·623	0·011

*After partial pancreatectomy; †comparing the means of the two groups.

Table 2 *Clinical changes during diazoxide treatment*

Case No	*Weight gain (g/kg/day)*	*Intake (ml/kg/day)*	*Pulse/minute*		*Respiration/minute*		*Heart murmur*	*Period (days)*
			Before treatment	*During treatment*	*Before treatment*	*During treatment*		
Toxic infants:								
1	−7·0	144	130	180	57	75	3/6	4
1*	21·8	150	140	190	44	66	3/6	4
2	3·4	152	154	158	56	83	3/6	3
3	17·6	215	134	170	48	54	2/6	16
4	6·0	168	150	168	47	56	2/6	2
5	25·4	220	146	160	49	63	3/6	2
6	3·0	193	120	155	47	66	2/6	9
7	10·1	172	155	165	46	64	3/6	7
Mean (SD)	10·0 (10·9)	177 (29·5)	141 (12·4)	168 (11·8)	49 (4·7)	66 (9·5)		5·9 (4·8)
Infants who were not toxic:								
2*	8·3	194	142	140	45	40	None	7
3*	2·0	131	126	130	35	39	None	16
4	7·56	164	144	140	51	50	None	7
Mean (SD)	6·0 (3·4)	163 (31·5)	137 (9·9)	137 (5·8)	44 (8·1)	43 (6·1)		10 (5·2)
p Value†	0·374	0·559	0·626	0·001	0·377	0·005		0·317

*After partial pancreatectomy; †comparing the means of the two groups.

indexes of 1533 and above developed symptoms of toxicity. When we compared infants with indexes of more than 1500 with those who had indexes of less than 1500 the difference was significant (p=0·006). The diazoxide dose was similar in both groups, (p=0·62).

In all the infants with symptoms of toxicity, cardiomegaly and heart murmurs (grade 2/6–3/6) developed (table 2). There was no significant difference in the heart rate before diazoxide treatment in the two groups. In comparison, the heart rate differed significantly in the two groups during treatment (p<0·001). The heart rate was similar before and during diazoxide treatment in infants without complications, though it differed significantly in infants with complications (p<0·01).

The respiratory rate was similar in both groups before diazoxide treatment and did not change during treatment in the group who did not become toxic. There was a significant increase in the respiratory rate during diazoxide treatment in the group who became toxic (p<0·01). Two infants, (cases 1 and 5) required mechanical ventilation with oxygen requirements of up to 100%.

Echocardiograms were done in patients 1, 5, and 7 and did not show any structural lesion. Cardiorespiratory symptoms resolved in all infants within 2–4 days after diazoxide was discontinued. Digoxin was not used. Chlorothiazide was given in conjunction with diazoxide, and frusemide was given as needed.

The average fluid intake and weight gain of the two groups of infants during treatment did not differ.

Temperatures between 36·5°C and 38·5°C were noticed in four infants who became toxic. A picture mimicking sepsis was also seen, and antibiotics were given to five infants. In none of these infants was any micro-organisms grown from blood, urine, or cerebrospinal fluid. Symptoms of central nervous system impairment including fussiness, irritability, jitterness, and diaphoresis were seen in five infants without hypoglycaemia. Three infants had seizure activity during diazoxide treatment that required anticonvulsant treatment. Blood glucose, calcium, magnesium, and electrolyte concentrations were normal when these episodes occurred.

Discussion

Hypoglycaemia in infants with persistent neonatal hyperinsulinism often requires various management regimens despite a high glucose infusion rate. Diazoxide in various doses had been used in combination with other drugs to control hypoglycaemia.[3-5] We have presented seven patients with persistent neonatal hyperinsulinism who received diazoxide in varying doses to control the blood glucose, and congestive heart failure occurred in all seven patients during eight treatment periods. The cause of the heart failure in similar infants is not clear from the few reported cases.[4-6]

Fluid intake was within the normal range, and there was no significant difference in the fluid intake between those who became toxic and those who did not. The weight gain during diazoxide treatment was within the normal range, and did not differ between the groups. All patients were on appropriate doses of chlorothiazide during diazoxide treatment. Our findings confirm those of McGraw and Price, who found no appreciable weight gain in a patient with nesidioblastosis who developed heart failure while receiving diazoxide treatment.[4] Heart failure resolved gradually in all patients within two to four days of discontinuing diazoxide. In view of these findings it is unlikely that fluid overload was the cause for the heart failure in these infants. A direct toxic effect of diazoxide on the myocardium seems to be a more probable explanation.

Poor myocardial function in hyperinsulinaemic states such as in infants of diabetic mothers has been well described.[8] None of our patients had structural heart disease or signs of heart failure before diazoxide treatment was started, and the heart failure that developed during diazoxide treatment resolved in all patients after the diazoxide was stopped. None of our patients received digoxin, and the rationale was that these patients have a hyperinsulinaemic state, and the myocardial state and function are most likely to be similar to those of infants of diabetic mothers.[8 9]

High doses of diazoxide (up to 20–25 mg/kg/day) have been recommended in an attempt to avoid operation, and heart failure had been attributed to the use of these high doses.[3–5 10] In our study there was no significant difference in diazoxide dose in infants with complications and those without. Our patients who developed heart failure, however, had high blood insulin and low blood glucose concentrations compared with those who did not develop complications. The insulin:glucose ratio was higher in those with, compared with those without, heart failure. These findings support the hypothesis that the more severe the disease state—even in the same patient—the more susceptible that patients is to the toxic effects of diazoxide. The diazoxide toxicity index was used in this study as a predictor of susceptibility to cardiorespiratory failure by combining the effect of diazoxide dose and the severity of the hyperinsulinaemic state. Our results show that toxicity symptoms occurred in a patient who received diazoxide in a dose as low as 12 mg/kg/day and did not occur in a patient who received a dose as high as 21 mg/kg/day.

All infants with an insulin:glucose ratio of more than 64 (more severe disease) and a toxicity index of more than 1533 developed symptoms of toxicity, and all infants with an insulin:glucose ratio of less than 32 (less severe disease) and a toxicity index of less than 675 did not. It seems that an insulin:glucose ratio above 48 and a toxicity index above 1104 (mid points between the lowest values of infants who became toxic and the highest values for infants who did not are values above which symptoms of toxicity are likely to occur. If diazoxide has to be used, a probable safe dose can be calculated if the insulin: glucose ratio is known, by dividing 1104 by the insulin glucose:ratio, with a maximum dose of 25 mg/kg/day.

Some other serious complications were observed in conjunction with diazoxide. Convulsions and other central nervous system symptoms that were noticed in our patients cannot be attributed solely to diazoxide, as hypoglycaemia is known to cause these symptoms, and ataxia has been reported by McGraw and Price in a patient with nesidioblastosis on diazoxide treatment.[4] A clinical picture mimicking sepsis that required treatment with antibiotics is another problem that may mislead the physician to institute unnecessary investigations and treatment. As in some previous reports,[11 12] all our patients except one required 95% to total pancreatectomy to control the blood glucose.

In summary, serious complications including cardiorespiratory failure were encountered during diazoxide treatment of seven infants with persistent neonatal hyperinsulinism. Cardiorespiratory failure was closely related to both the diazoxide dose and the severity of the disease. Further studies including measurements of the indices of left ventricular function will help to clarify further the cardiotoxic effect in such patients. By using the toxicity index it may be possible to predict which patients are likely to develop cardiorespiratory failure during diazoxide treatment.

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References

[1] Yakovac WC, Baker L, Hummeler K. Beta cell nesidioblastosis in idiopathic hypoglycaemia of infancy, *J Pediatr* 1971;**79**:226–31.

[2] Heitz PU, Kloppel G, Hacki WH, Polak JM, Pearse AGE. Morphologic and quantitative analysis of seven cases based on specific immunostaining and electron microscopy. *Diabetes* 1977;**26**:632–42.

[3] Aynsley-Green A, Polak JM, Bloom SR, *et al.* Nesidioblastosis of the pancreas: definition of the syndrome and the management of the severe neonatal hyperinsulinaemic hypoglycaemia. *Arch Dis Child* 1981;**56**:496–508.

[4] McGraw ME, Price DA. Complications of diazoxide in the treatment of nesidioblastosis. *Arch Dis Child* 1985;**60**:62–4.

[5] Gillies DR. Complications of diazoxide in the treatment of nesidioblastosis. *Arch Dis Child* 1985;**60**:500–1.

[6] Abu-Osba YK, Young M. Cardiorespiratory failure secondary

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Dystrophin analysis in the diagnosis of muscular dystrophy

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SUMMARY We present a family in which the differential diagnosis between X linked Duchenne muscular dystrophy and autosomal recessive Duchenne-like muscular dystrophy was resolved in favour of the latter by analysis of dystrophin, which is the protein product of the Duchenne muscular dystrophy locus.

X linked Duchenne muscular dystrophy is the commonest of the neuromuscular diseases of childhood, occurring in approximately 1 in 3300 live male births.[1] In contrast, autosomal recessive Duchenne-like muscular dystrophy is rare.[2 3] We describe a family where the differential diagnosis lay between these two conditions, and was resolved by analysis of dystrophin, which is the protein product of the Duchenne muscular dystrophy locus.[4]

Case reports

The family was referred for genetic counselling because the eldest daughter (DS), now aged 13 years, had a diagnosis of muscular dystrophy. The parents were healthy and unrelated; there was no family history of muscle disease. They also had a son of 2 years (JS) and a normal daughter aged 10 years.

CASE 1

DS toe walked from the age of 10 months, suffered noticeable difficulty walking at age 5 years, and by the next year had trouble climbing stairs. Muscle strength continued to deteriorate slowly and she was able to walk but unable to rise from the lying to standing position unaided at 12 years. On examination by one of us (HEH) there was proximal muscle weakness and wasting affecting both limb girdles and hypertrophy of both calves. Serum creatine kinase activity at age 9 years was 1500 IU/l and a muscle biopsy specimen taken at the same age showed a necrotising myopathy compatible with muscular dystrophy but without a focal pattern of distribution of the necrotic muscle fibres. Electrocardiography was normal at 13 years. Her karyotype was normal 46XX by high resolution banding.

CASE 2

JS, now aged 2 years, was late in walking at 18 months. He was slightly awkward when running and climbing stairs, and grasped furniture in order to rise from the floor. On examination there was slight weakness of latissimus dorsi, and mild enlargement of both calves, but muscle strength was otherwise normal. His serum creatine kinase activity at 21 months was 4526 IU/l. Electrocardiography was normal. A muscle biopsy specimen showed extensive areas of normal muscle fibres, but with small groups of fibres showing some atrophy and considerable basophilia suggesting focal regeneration. There were evenly scattered hyaline fibres and no excess of fat or connective tissue or disruption of muscle architecture. The histochemical profile was normal. These appearances were considered to be more in favour of autosomal recessive muscular dystrophy than Duchenne muscular dystrophy because of the focal distribution and lack of excess connective tissue.

INVESTIGATIONS

Moser and Emery suggested that manifesting carriers of Duchenne muscular dystrophy are as common as patients with 'limb-girdle dystrophy', at least in adults.[5] Because of her calf hypertrophy we were concerned that DS might be a manifesting carrier of Duchenne muscular dystrophy. Furthermore her brother had clinical features consistent with Duchenne muscular dystrophy and there was some evidence that he might be more severely affected. Autosomal recessive Duchenne-like muscular dystrophy is rare and some possible cases have been rediagnosed as X linked Duchenne muscular dystrophy.[3 6] Accordingly, we screened DNA from JS with dystrophin cDNA probes cDNA

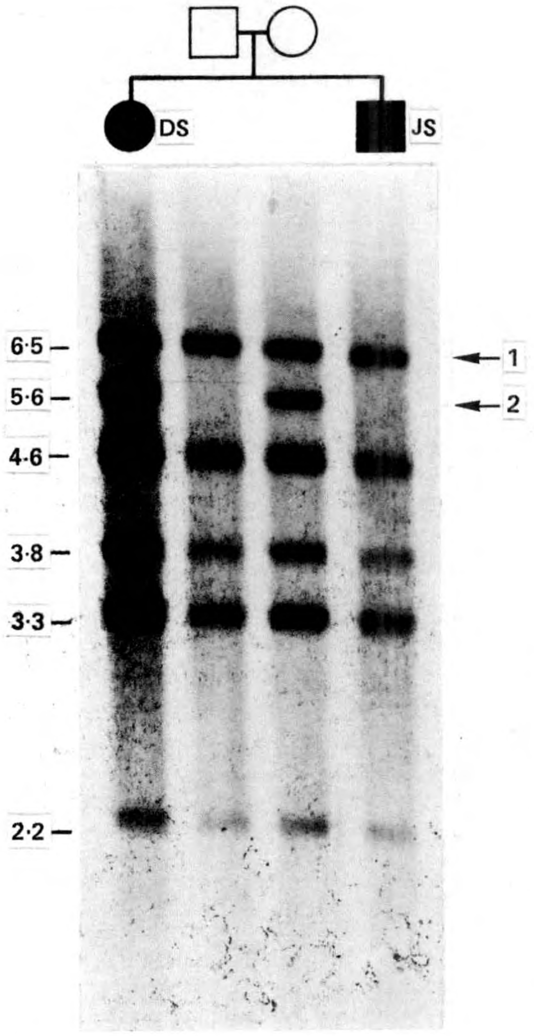

Fig 1 *TaqI digest of DNA probed with cDNA 8. There is a polymorphism with two alleles as arrowed. Allele 1 is 6·5 kb and allele 2 is 5·6 kb in length. The mother of DS and JS is heterozygous. DS has inherited allele 2 and JS allele 1. This would be expected in 5% of families with Duchenne muscular dystrophy where the site of mutation is unknown.*

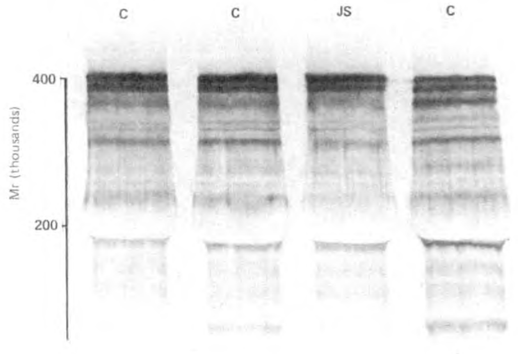

Fig 2 *Western blot analysis of dystrophin in control (C) and patient (JS) muscle samples. Binding of dystrophin monoclonal antibody was visualised with horseradish-peroxidase labelled second antibody exposed to diaminobenzidine. Relative molecular mass (Mr) measurements are approximate.*

1–3 and cDNA 6–8, which detect molecular deletions in at least 60% of boys with Duchenne muscular dystrophy.[7 8] No such deletion was found. Several polymorphisms have been described with these probes.[7 8] However, we only found the mother of JS to be heterozygous (and thus informative) with the cDNA 8/Taq1 polymorphism: JS and DS had inherited opposite alleles (fig 1), which would be expected in only 5% of families with Duchenne muscular dystrophy.

METHODS

Dystrophin analysis was performed by homogenising small samples of muscle in sodium dodecyl sulphate polyacrylamide gel treatment buffer and separating the extracted proteins on a 4–8% gradient slabgel.[9] After electrophoretic transfer[10] of the proteins to nitrocellulose paper (western blotting) the samples were probed with a specific monoclonal antibody to dystrophin.[11] The antibody produces a characteristic pattern of bands from muscle of normal control individuals and the result from the muscle sample from JS was indistinguishable from these controls (fig 2).

Discussion

This family highlights the difficulty in distinguishing between the rare manifesting carrier of the relatively common Duchenne muscular dystrophy gene, and the rare autosomal recessive Duchenne-like muscular dystrophy. Although the latter form of muscular dystrophy is well documented in North Africa and Arabia,[12 13] it is very uncommon among Europeans.[2 3 14] The diagnosis could not be resolved by traditional methods. Although muscle histology favoured autosomal recessive dystrophy, this interpretation is not absolute, and Duchenne muscular dystrophy remained a possible diagnosis on clinical grounds. DNA analysis with dystrophin cDNA probes did not support but could not exclude Duchenne muscular dystrophy in this family. Dystrophin analysis showed no abnormality in the affected boy, however, whereas abnormalities are found in at least 97% of boys with Duchenne muscular dystrophy.[15] Therefore we conclude that this family is a genuine example of autosomal recessive Duchenne-like muscular dystrophy and counselled them accordingly. It is possible that the one Duchenne muscular dystrophy boy without dystrophin abnormality in the study of Hoffman *et al* may also have this diagnosis.[15]

This family represents one of the few proved European families reported with autosomal recessive Duchenne-like muscular dystrophy and to our knowledge there has been no previous report of dystrophin analysis being used to resolve this problem.

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References

[1] Emery AEH. *Duchenne muscular dystrophy.* Oxford monograph on medical genetics. Harper P. Bobrow M. eds. No 15. Oxford: Oxford University Press, 1987.

[2] Somer H. Voutilainen A. Knuutila S. Kaitila I, Rapola J, Leinonen H. Duchenne-like muscular dystrophy in two sisters with normal karyotypes: evidence for autosomal recessive inheritance. *Clin Genet* 1985;**28:**151–6.

[3] Gardner-Medwin D, Johnston HM. Severe muscular dystrophy in girls. *J Neurol Sci* 1984;**64:**79–87.

[4] Hoffman EP, Brown RH, Kunkel LM. Dystrophin: the protein product of the Duchenne muscular dystrophy locus. *Cell* 1987;**51:**919–28.

[5] Moser H, Emery AEH. The manifesting carrier in Duchenne muscular dystrophy. *Clin Genet* 1974;**5:**271–84.

[6] Francke U, Darras BT, Hersh JH, Berg B, Miller R. Brother/sister pairs affected with muscular dystrophy: molecular studies reveal etiologic heterogeneity. *Am J Hum Genet* 1988;**43:**A83.

[7] Read AP, Mountford RC, Forrest SM, Kenwrick SJ, Davies KE, Harris R. Patterns of exon deletions in Duchenne and Becker muscular dystrophy. *Hum Genet* 1988;**80:**152–6.

[8] Darras BT, Blattner P, Harper JF, Spiro AJ, Alter S, Francke U. Intragenic deletions in 21 Duchenne muscular dystrophy (DMD)/Becker muscular dystrophy (BMD) families studied with the dystrophin cDNA: location of breakpoints on HindIII and BglII exon-containing fragment maps, meiotic and mitotic origin of the mutations. *Am J Hum Genet* 1988;**43:**620–9.

[9] Laemmli VK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;**227:** 680–5.

[10] Otter T, King SM, Witman GB. A two-step procedure for efficient electrotransfer of both high molecular weight (>400 000) and low molecular weight (<20 000) proteins. *Ana Biochem* 1987;**162:**370–7.

[11] Nicholson LVB, Davison K, Falkous G, *et al.* Dystrophin in skeletal muscle. Part I. Western blot analysis using a monoclonal antibody. *J Neurol Sci* (in press).

[12] Hamida MB, Fardeau M, Attia N. Severe childhood muscular dystrophy affecting both sexes and frequent in Tunisia. *Muscle Nerve* 1983;**6:**469–80.

[13] Salih MAM, Omer MIA, Bayoumi RA, Karrar O, Johnson M. Severe autosomal recessive muscular dystrophy in an extended Sudanese kindred. *Dev Med Child Neurol* 1983;**25:**43–52.

[14] Gardner-Medwin D, Clarke A, Sharples P. Some studies of the Duchenne and autosomal recessive types of muscular dystrophy. *Brain Dev* 1989;**11:**91–7.

[15] Hoffman EP, Fischbeck KH, Brown RH, *et al.* Characterisation of dystrophin in muscle-biopsy specimens from patients with Duchenne's or Becker's muscular dystrophy. *N Engl J Med* 1988;**318:**1363–8.

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Paracetamol suppositories: a comparative study

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SUMMARY Paracetamol suppositories in two different bases were given to children who had fever after operations. Plasma concentrations and the effect on temperature were compared. There was a significant correlation between peak plasma concentrations and maximum drop in temperature. A lipophilic base produced better results than a hydrophilic base.

Paracetamol given orally is standard treatment for fever. A problem arises, however, with children with impaired consciousness or who have nausea or vomiting. Until recently aspirin suppositories have met this need.[1] Although there have been no reports of Reye's syndrome associated with aspirin given rectally, it would seem prudent to avoid it. Many studies have documented the efficacy of paracetamol given orally for the treatment of fever.[2] Although clinical studies have shown that paracetamol given rectally is also effective, there is little information about its pharmacokinetics in children.[3]

Patients and methods

Twenty eight children recovering from cardiac operations were studied. Their ages ranged from 2 months to 8·3 years. All patients received the drug on the first day after operation. Initially 18 children were given 15–20 mg/kg paracetamol in a hydrophilic base as a single dose. The mean (SD) initial temperature in this group was 38·9 (0·6)°C. After review of the results a further 10 patients were given the same dose in a lipophilic base. The mean initial temperature in this group was 39·1 (0·7)°C.

Blood was taken at 0, 30, 60, 120, and 180 minutes for measurement of paracetamol concentrations. One ml of blood from the intravenous line was placed in a lithium heparin container and refrigerated until assayed. Plasma paracetamol concentrations were measured by a modified enzymatic technique on a Cobas Fara analyser as described by Kenny and Ward.[4] Rectal and peripheral temperatures were recorded routinely for each patient. The study protocol was approved by the hospital ethics and research committees.

Results

The plasma paracetamol concentrations achieved by the two preparations are shown in fig 1. There were significant correlations between the peak plasma paracetamol concentration and the maximum decrease in temperature achieved, the values for the

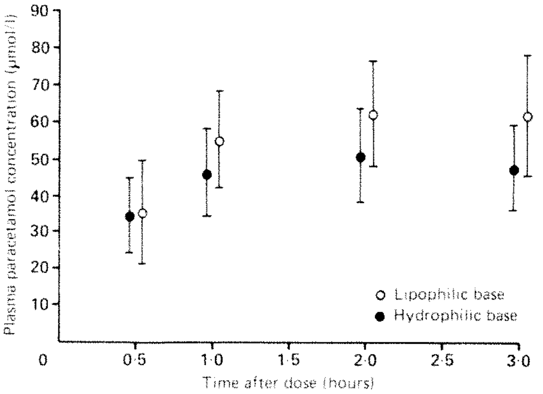

Fig 1 *Mean (SEM) plasma paracetamol concentrations.*

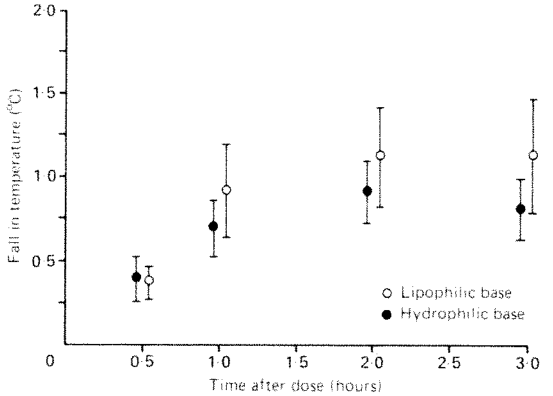

Fig 2 *Mean (SEM) fall in temperature after paracetamol suppositories had been given.*

lipophilic base and the hydrophilic base being 0·63 and −0·56, respectively.

The peak plasma concentrations achieved by paracetamol in a lipophilic base were significantly higher than in the group receiving the hydrophilic preparation ($p<0{\cdot}03$, Wilcoxon rank sum test) despite variation in the time taken to achieve these concentrations. The maximum decrease in temperature achieved by the paracetamol in the lipophilic base was also significantly greater ($p<0{\cdot}037$, Wilcoxon rank sum test) but once again this occurred at varying time intervals (fig 2). In those who received paracetamol in a hydrophilic base the mean drop in temperature was −1·0°C (range −0·4 to −1·7°C). The temperature rose again between three and four hours later. In the second group (who received paracetamol in a lipophilic base), the mean decrease in temperature was −1·5°C (range −0·6 to −2·2°C); this reduction in temperature lasted longer.

Discussion

Studies in adults have shown that maximum plasma concentrations after oral paracetamol are reached within 15 to 120 minutes, depending on the time since the last meal. The reported time for peak plasma concentrations to be reached after rectal administration was significantly longer with a range of 60 to 240 minutes.[5] Our results compare favourably with these reports.

The formulation of the base has been shown to have a profound effect on the adsorption of poorly soluble drugs such as paracetamol. Other studies have documented the superiority of triglyceride bases in increasing rectal absorption of paracetamol.[6]

We found no correlation between the age of the patient and either peak plasma concentration or maximum drop in temperature.

Although there was a wide range and considerable overlap in both the plasma paracetamol concentrations and the reductions in temperature achieved at the various time intervals, the lipophilic based paracetamol proved to be superior on average producing higher peak plasma concentrations and a better therapeutic response. We realise, however, that this conclusion can be criticised on the basis that the two groups of patients were not randomised.

Clinical trials have confirmed the efficacy of rectal paracetamol in children. We believe that paracetamol suppositories are effective in reducing fever whenever an alternative to oral medication is required.

We thank Rice Steele and Co who prepared the suppositories, and the nursing staff of the intensive care unit for recording the observations.

References

[1] Connolly K, Lam L, Ward OC. Rectal aspirin—absorption and antipyretic effect. *Arch Dis Child* 1954:713–4.

[2] Lovejoy F. Aspirin and acetaminophen: a comparative view of their antipyretic and analgesic activity. *Pediatrics* 1978;**62**:904–9.

[3] Vernon S, Bacon C, Weightmann D. Rectal paracetamol in small children. *Arch Dis Child* 1979;**5**:469–70.

[4] Kenny D, Ward J. Determination of therapeutic concentrations of paracetamol in serum with the Cobas Fara analyser. *Ann Clin Biochem* 1987;**24**(suppl):1–73.

[5] Paulsen H, Kreilgard B. Bioavailability of paracetamol after single oral and rectal administration. *Archives of Pharmaceutical Chemistry Sciences Edition* 1984;**12**:97–102.

[6] Moolenar F, Schooner AJM, Everts A, Huizinga T. Absorption rate and bioavailability from fatty suppositories. *Pharmaceutisch Weekblad Sciences Edition* 1979;**112**:89–94.

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Personal practice

Dietary treatment of atopic eczema

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Atopic eczema is merely a skin disease to doctors, but to children it can be a disfiguring handicap. Conventional treatment comprises the use of emollients, steroids, sedating antihistamines at night, antibiotics, bandaging, and the avoidance of irritant detergents or clothing.[1] Even with the most devoted application of these measures, there remain a number of children with persistent and troublesome disease. This paper examines the role for elimination diets. The subject is controversial, a legacy of the serious lack of objective data.

Evidence from clinical studies

There are no double blind studies in children with eczema comparing dietary therapy with other treatment, and given the cooperation required with diets it is difficult to see how it would be possible to apply a diet in a truly blind manner. Absence of such studies is serious, for without them it is impossible to tell whether diets are simply of temporary benefit (until the child grows out of the disease) or whether such treatment actually influences the ultimate prognosis by shortening the duration of the disease. There is also a lack of published data about the long term results of different elimination diets. A starting point are the following established facts:

(1) Double blind challenge studies have established that atopic eczema can be a feature of cows' milk protein intolerance. The incidence of eczema in patients with cows' milk protein intolerence depends on how they are selected, but lies between 10% and 40%.[2 3]

(2) Studies of double blind food challenges have established that in selected children with atopic eczema, a number of foods in doses of 8 g or less can provoke an immediate (within two hours) itchy erythematous macular rash, accompanied by a significant rise in the plasma histamine concentration.[4 5] There are two problems with these types of data. Firstly, implicit in these studies is the inference that these erythematous rashes would lead to the development or worsening of eczema if further doses of challenge material were given, but this has not been proved. Challenges were given as little as five hours apart, precluding the observation of delayed onset reactions or worsening of eczema. The conventional dermatological view in Britain is that these immediate erythematous reactions are indeed common in children with atopic eczema but represent an entirely separate and unrelated problem.[6] Secondly, a single provocation with a small dose in the laboratory differs greatly from the real life situation of continuous or repeated exposure to larger doses of foods. For example, Hill found that whereas 8 to 10 g of cows' milk powder (corresponding to 60 to 70 ml of milk) was adequate to provoke a response in some patients with cows' milk protein intolerance, other patients (with late onset symptoms and particularly eczema[3]) required up to 10 times this volume of milk daily for more than 48 hours before symptoms developed.[7]

(3) There are several studies in which children with atopic eczema improved on an elimination diet and were then challenged openly or double blind with single foods.[8-10] The drawback to this study design is that positive double blind challenges can confirm the presence of food intolerance but do not establish how much improvement is attributable to food avoidance and how much is due to a placebo effect.

Patient selection

Elimination diets are easy to prescribe but difficult to follow. By preventing a child from eating food consumed by family or friends a diet can itself be a handicap, and the emotional consequences of such treatment have never been studied. Elimination diets are costly,[11] and even the most highly motivated family may be unable or unwilling to comply with the diet despite improvement in the eczema.[9]

In view of the relative complexity of diets, it makes no sense to use a diet where simple and safe topical treatment will suffice. Local experience is that a large number of children referred with eczema have unrecognised but simply treatable problems such as bacterial infection,[12] dry ichthyotic skin,[1] or long dirty fingernails, for which dietary treatment would be irrelevant.

Skin prick tests and radioallergosorbent (RAST) tests are not helpful because of the high rate of false positive[13] and false negative[14] results. Neither test can indicate which patient will respond to a diet, or which foods should be eliminated.[15]

Objective data from which one could derive recommendations for patient selection are unavailable. Based on local treatment and follow up, the best indications for trial of an elimination diet are:

(1) Widespread (for example, affecting 15% or more of the skin surface area) atopic eczema unresponsive to topical treatment.

(2) Patient aged under 12 months, partly because elimination diets are simpler to administer and control in infancy,[9] and also because it is a general observation that results are better in this age group.[15]

(3) Patient with symptoms in other systems, particularly persistent loose stools, or other atopic disorders such as asthma. It is far less common for food intolerance to cause a single symptom (for example, eczema) than multiple symptoms (for example, eczema, asthma, loose stools, and vomiting). In one study where selected children with atopic eczema received double blind food challenges, gastrointestinal symptoms (vomiting, loose stools, abdominal pain) occurred in 52% of positive challenges, and respiratory symptoms (asthma, stridor, sneezing) occurred in 32% of positive challenges.[4]

(4) Severe eczema in exclusively breast fed infants. In one study, in six of 37 breast fed infants, eczema improved when the mother avoided cows' milk protein and egg and relapsed when these were reintroduced.[16] It was impossible to predict which baby would respond to maternal dietary exclusion, and it is reasonable to try maternal avoidance of cows' milk in an infant with eczema who is being exclusively breast fed. Other foods can provoke eczema in this way, but their detection depends on parental suspicion followed by avoidance and challenge.

(5) Parents keen to try a diet. Where eczema is widespread and unresponsive to simple treatment, and where the parents are keen to try food elimination, it makes sense to try a diet with proper control and supervision rather than driving the family into the hands of an unorthodox therapist.[17]

Elimination diet procedure

The fundamental strategy is to try an elimination diet for a defined period of time. Six weeks is long enough to ensure that any improvement will not have been missed, while also allowing for the inevitable day to day fluctuations in disease activity. At the end of this period the patient is assessed by the same person who saw the patient at the beginning of the diet, and the diet abandoned if there has not been much improvement. Parents (and children) are sometimes so keen that a diet should succeed that they perceive improvement where none exists, and they need the help of an objecfive observer. Otherwise there is a risk that a useless diet will be continued indefinitely.

If the diet has clearly helped, and the parents and child wish to continue, then the next step is to try to identify food triggers by the reintroduction of foods singly at the rate of one new food every five to seven days. Foods which are tolerated are left in the diet. The presence of food intolerance is suggested by worsening of the disease after introduction of a food, followed by improvement when the food is stopped. Failure of the disease to improve when a new food is stopped suggests an alternative cause for the worsening. The identification of food triggers is far from easy because of confounding variables such as the normal daily fluctuation in disease activity, intercurrent infections (which may worsen or improve eczema), exposure to other triggers (for example, pets), or teething. There is a small risk of anaphylaxis occurring with food reintroductions, particularly with cows' milk and with patients on elemental diets.[18]

Supervision by a dietician is required, for without it there is a risk that the diet will fail because specific food items have not been fully excluded. Elimination diets carry the risk of nutritional inadequacy,[19] even where a cows' milk substitute has been prescribed, and is another reason for dietetic support.[20]

Local experience is that in atopic eczema the incidence of allergy to household pets and house dust mites is at least as common, if not more so, than food intolerance. It is logical to consider attempts to avoid these in conjunction with a diet, as a diet may fail in the face of continued exposure to pets or dust mites. As with foods, tests cannot predict the outcome of environmental allergen avoidance.

Types of diet

(1) HALF HEARTED ATTEMPTS TO 'HAVE A GO'

The very small quantity of food which can provoke an adverse reaction means that the 'try cutting down his milk' type of tinkering with the diet is most unlikely to succeed. The advantage of a carefully conducted diet is that even if it fails at least the parents will be satisfied in the knowledge that it was tried properly.

(2) COMPLETE AVOIDANCE OF KNOWN TRIGGERS

A trial of rigorous avoidance of known or suspected triggers is a logical first step. It is common to see a child with a clear history of intolerance to a food, but where the food is being incompletely avoided. An example would be a child with a history suggesting cows' milk protein intolerance who is avoiding cows' milk but consuming products which contain whey or casein, or who is receiving goats' milk. (The appreciable antigenic similarity between cows' and goats' milk proteins [21] suggests that goats' milk is unlikely to be tolerated in most children with genuine cows' milk protein intolerance.)

(3) COWS' MILK PROTEIN AND EGG AVOIDANCE

Trials of this treatment have given conflicting results.[15 22] Local experience is that less than 20% of patients benefit, and the main place for this diet is in infancy.

(4) ELIMINATION OF COMMON POSSIBLE TRIGGERS

The patient avoids foods for which there is a history of intolerance, plus approximately 10 common food triggers such as cows' milk, egg, wheat, fish, legumes (pea, bean, soya, lentil), tomato, nuts, berries and currants, citrus fruit, and food additives (azo dyes, benzoates, and sulphites). Local experience is that less than 20% experience a useful clinical benefit.

(5) THE FEW FOOD DIET

This consists of exclusion of all foods except for five or six items.[23] Such diets comprise a meat (usually lamb or turkey), three vegetables (for example, potato, rice, and carrot or a brassica —cauliflower, cabbage, broccoli, or sprouts), a fruit (usually pear) and possibly a breakfast cereal (for example Rice Crispies). Local experience is that in children under the age of 5 years with 25% or more skin surface affected, approximately a quarter can be expected to usefully improve on the diet.

(6) ELEMENTAL DIET

The application of an inpatient regimen of four to six weeks of a so called elemental diet (for example, Vivonex, Norwich Eaton Labortories) is the ultimate test of whether food intolerance is relevant or not, but until more data are available this approach must be regarded as experimental. Based on unpublished local experience, the drawbacks comprise the lack of a guarantee of success, family disruption associated with two to three month's hospitalisation, and sometimes loose stools (due to hyperosmolarity of the diet), severe weight loss, and profound hypoalbuminaemia.

Conclusions

Like many chronic diseases, there is a strong placebo effect of any new treatment applied with enthusiasm, particularly if the parents are convinced it will help. Long term follow up of patients seen locally and elsewhere suggests, however, that probably no more than 10% of children with atopic eczema severe enough to warrant referral to hospital are likely to derive lasting benefit from the use of an elimination diet.[15] As diets are difficult and potentially hazardous, and are possibly best employed in conjunction with pet and dust mite avoidance, it is logical to apply simpler conventional treatment first. Despite the somewhat disappointing overall results of diets, a number of patients with severe disease experience dramatic and lasting benefit from specific food avoidance, and the detection of these children by the application of empirically based diets is an important part of the management of eczema.

References

[1] David TJ. The practical management of atopic eczema in childhood. *Maternal and Child Health* 1986;**11:**60–4.

[2] Goldman AS, Anderson DW, Sellers WA, Saperstein S, Kniker WT, Halpern SR. Milk allergy. I. Oral challenge with milk and isolated milk proteins in allergic children. *Pediatrics* 1963;**32:** 425–43.

[3] Hill DJ, Ford RPK, Shelton MJ, Hosking CS. A study of 100 infants and young children with cows' milk allergy. *Clin Rev Allergy* 1984;**2:**125–42.

[4] Sampson HA, McCaskill CC. Food hypersensitivity and atopic dermatitis: evaluation of 113 patients. *J Pediatr* 1985;**107:** 669–75.

[5] Sampson HA, Jolie PA. Increased plasma histamine concentration after food challenges in children with atopic dermatitis. *N Engl J Med* 1984;**311:**372–6.

[6] Allen R. Role of diet in treating atopic eczema: dietary manipulation has no value. *Br Med J* 1988;**297:**1459–60.

[7] Hill DJ, Ball G, Hosking CS. Clinical manifestations of cows' milk allergy in childhood. I. Associations with in-vitro cellular immune responses. *Clin Allergy* 1988;**18:**469–79.

[8] Hill DJ.Lynch BC. Elemental diet in the management of severe eczema in childhood. *Clin Allergy* 1982;**12:**313–5.

[9] Hathaway MJ, Warner JO. Compliance problems in the dietary management of eczema. *Arch Dis Child* 1983;**58:**463–4.

[10] Van Asperen PP, Lewis M, Rogers M, Kemp AS, Thompson S. Experience with an elimination diet in children with atopic dermatitis. *Clin Allergy* 1983;**13:**479–85.

[11] Macdonald A, Forsythe WI. The cost of nutrition and diet therapy for low-income families. *Hum Nutr Appl Nutr* 1986; **40A:**87–96.

[12] David TJ, Cambridge GC. Bacterial infection and atopic eczema. *Arch Dis Child* 1986;**61:**20–3.

[13] Sampson HA, Albergo R. Comparison of results of skin tests, RAST, and double-blind, placebo-controlled food challenges in children with atopic dermatitis. *J Allergy Clin Immunol* 1984;**74:** 26–33.

[14] Hill DJ, Duke AM, Hosking CS, Hudson IL. Clinical manifesta-

tions of cows' milk allergy in childhood. II. The diagnostic value of skin tests and RAST. *Clin Allergy* 1988;**18**:481–90.

[15] Atherton DJ. Diet and atopic eczema. *Clin Allergy* 1988;**18**:215–28.

[16] Cant AJ, Bailes JA, Marsden RA, Hewitt D. Effect of maternal dietary exclusion on breast fed infants with eczema: two controlled studies. *Br Med J* 1986;**293**:231–3.

[17] David TJ. Unhelpful recent developments in the diagnosis and treatment of allergy and food intolerance in children. In: Dobbing J, ed. *Food intolerance*. London:Baillière Tindall, 1987:185–214.

[18] David TJ. Anaphylactic shock during elimination diets for severe atopic eczema. *Arch Dis Child* 1984;**59**:983–6.

[19] David TJ, Waddington E, Stanton RHJ. Nutritional hazards of elimination diets in children with atopic eczema. *Arch Dis Child* 1984;**59**:323–5.

[20] Devlin J, Stanton RHJ, David TJ. Calcium intake and cows' milk free diets. *Arch Dis Child* 1989;**64**:1183–4.

[21] Crawford LV, Grogan FT. Allergenicity of cows' milk proteins. IV. Relationship of goats' milk proteins as studied by serum-agar precipitation. *J Pediatr* 1961;**59**:347–50.

[22] Neild VS, Marsden RA, Bailes JA, Bland JM. Egg and milk exclusion diets in atopic eczema. *Br J Dermatol* 1986;**114**:117–23.

[23] Thomas B, ed. Atopic eczema (infantile eczema, dermatitis). *Manual of dietetic practice*. Oxford: Blackwell, 1988:527–31.

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Dr Clement A Smith

Recently, at the age of 87, Dr Clement Smith died peacefully at his home in Cambridge, Massachusetts. In the 1940s and 50s it was he more than anyone else who set the scientific tone for the new speciality of neonatal paediatrics. He made important contributions to the study of hyaline membrane disease relating to abnormalities in lung mechanics and to the associated metabolic disturbance attributable to tissue breakdown. It was. however, as a reviewer and critical commentator that he made his most important contributions. In particular his book, *Physiology of the Newborn Infant,* first published in 1945, served as a powerful advocate of the scientific approach to this subject. Among the 50 or so fellows who studied under Clem in the course of his research career, many came from European countries, including the United Kingdom. In this way he planted the seeds of his scientific curiosity in many places far removed geographically and spiritually from Boston.

Born and raised in Ann Arbor, he retained the pleasing style of a grown up Tom Sawyer. Mark Twain was certainly one of his many literary heroes. His early education included a master's degree in English Literature and he never lost his relish for the pleasures of reading and writing. His long career at Harvard Medical School began with a residency at Children's in 1931 and ended only with his last attendance at grand rounds in December 1988. From 1962–73 he was Editor of the important journal, *Pediatrics*; and in 1983 he published a history of The Boston Children's Hospital. Many of his pupils will remember with pleasure the good tempered wit, the modest courtesy, and the generous hospitality of this old fashioned American gentleman. His influence on the development of neonatal paediatrics will continue for many years.

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Contemporary history

David Morris—a paediatrician remembered

H R GAMSU

I should like to pay my respects with you to David the man and the paediatrician. I suppose one should start conventionally, though there was little that was conventional about David Morris. His irrepressibly brisk and friendly manner and his carefully chosen clothes immediately made him stand out in a crowd, and the causes he espoused and his tenacity in pursuing them singled him out as exceptional, and anything but conventional.

After qualifying at the Middlesex Hospital he began his training in paediatrics at the Queen Elizabeth Hospital for Children in Hackney and subsequently worked at St Andrew's Hospital in the East End. In 1952 he was made Consultant in Paediatrics at the British Hospital for Mothers and Babies in Woolwich and at the Brook General Hospital in Shooters Hill where he established the Children's Department and worked closely with Dr Ashad Warley in doing so. At Woolwich, he set about to extend the work of Dr Harold Waller and soon became involved in those issues concerning the mother and her infant which were to preoccupy him during the rest of his professional life.

Though as an active clinical paediatrician, his proper and main initial concern was for the physical welfare of his patients, and for the precept put forward by Denis Browne, a contemporary orthopaedic surgeon, that 'the first thing about the mother and baby relationship is to ensure that they both stay alive'. David certainly did not stop at that. Separation of the mother from her newborn baby, the lack of emphasis on breast feeding, the care of children in adult wards, restricted visiting of hospitalised children, and above all, the lack of the best facilities and attitudes in the care given to disabled children that considerably handicapped them further, were the issues that preoccupied him more and more. He determined early on to protect the interests of the most neglected children, those who were emotionally and socially deprived and the physically and mentally handicapped.

He began to work tirelessly to create the best facilities at Goldie Lee Nursery in his own district which catered for children with special needs. He was not only preoccupied with improving the physical facilities and conditions for these children, but he also endeavoured to see to it that the right people came to work with the children in need. David also set about to encourage people to adopt the right attitudes and to develop the ability to create the improvements that were most needed. In 1963 the first Save The Children Fund playgroup was established at Woolwich and from David's unit this extended to others. The importance of play in hospital was a matter that David made peculiarly his own but like so many of the innovations that he put forward, he made sure that his pleasure in the success of this project was shared with others so that the movement spread quickly.

David's ability to talk to children, to relate easily to them and to gain their confidence was unparalleled. One little boy said 'He is a nice doctor because he talks to me, not only to my mum and dad—and he can do hand-stands!'

He was funny and full of fun and inevitably drew children into responding similarly. He persuaded all of us to look at the whole child and not just at the

child's physical illness, and to regard the child as part of the family, not just as a sick individual. He was dedicated to helping parents to love and cherish their children: those who were disabled and those who were well.

David was a great communicator in many tongues. His fluency in French had led to his secondment during the war as a surgeon to the Free French Navy. He maintained and extended that fluency. He also made it his business to know a few welcoming phrases in many languages. So perfect was his pronunciation and so uninhibited his use of gesture, that not only did this make people he spoke to much more at ease, but also convinced them that he was prodigiously polyglot.

He made his junior staff feel important and made every effort to put them at their ease. He kept in touch with the progress of their careers and gave them every encouragement and help along the way. They always felt that here was someone they could come to with their troubles and be assured of good advice and reliable help.

David was honest enough to acknowledge that one could not know everything, and on appropriate occasions would refer a child with an especially difficult problem to one of his wide circle of friends and colleagues. One could be certain that he would select the best person, and parents and child patients would always be made to feel secure and reassured by the fact that David had chosen well and wisely.

He made best use of a multidisciplinary approach in patient care and worked closely with psychotherapists and social workers who were part of his team.

His many projects are almost too numerous to recount and even then I am sure that many of them are not known to me. I should like to mention only some that I have singled out as specially significant to me.

He established the Psychiatric Discussion Group for Paediatricians with Anna Freud at her home in Hampstead. A number of like minded people regularly came, including Ronnie McKeith who was a great friend and someone he recognised early on as being similarly motivated. This group included great names in paediatrics—Grahame Fagg, Dermod McCarthy, Tina Cooper, Bianca Gordon, Otto Woolf, Aidan McFarlane, Trevor Mann, and Anna Freud herself, and was even more famous outside this country, having attracted those paediatricians who were able to extend the role of child psychiatry to their own clinical practice overseas, for example, Marshall Klaus, Benjamin Spock, Henry Kempe, to name only a few. David was a founder member of the Balint Society and in his role as chairman and participant attended all the meetings and guided much of the discussion. He had a durable involvement with the Association of Child Psychiatrists and Psychologists, and in this last year as its chairman, and true to his long held tenets, he tried to open its doors to anyone whom he felt would be benefited. He also had a close relationship with the workers on Mencap and was a staunch advisor and committee member.

In this respect it is important to mention David's ability to seek out in every individual, professional, friend, patient, that person's best qualities and to provide the catalyst which would allow these qualities to develop. Someone who immediately comes to mind in this regard, though as far as I am aware not known to David as a patient, was Christopher Nolan whose mother had the perception to coax his great talent out of his disabled, severely handicapped body and cause it to be revealed. Christopher as you know is a talented writer and his poem written in 1980 called 'Nominating' I think characterises the sort of responses that perceptive people will seek even in people who seem less able to give them. This was a principle that David was very well aware of:

'Pity the man in failure,
Love the man who's mad,
Make music with the man that's happy,
Marvel at a lonesome-child's hand;
Outstretched in those innocent fingers
Is a lively benediction's grace,
Make careful study, make mercy chime,
Love the tear, dry the face, watch the smile'.

David's work with disadvantaged children is exemplified by many activities and particularly by the Uphill Ski Club which he founded. For many years he would accompany groups of handicapped children on a skiing jaunt which he would personally conduct and supervise. He was chairman of the Nordoff-Robbins Music Therapy Centre and chairman of the Forum on Mental Retardation which he established at the Royal Society of Medicine. He was a member of the Medical Advisory Panel on Mentally Handicapped Children and remained active in this long after his retirement. In fact the British Council recognising his expertise asked him to visit a number of countries including Cyprus and Greece and countries in South America to advise on the creation of facilities for the handicapped.

David made his mark on the international and national paediatric scene. He regularly attended the annual meetings of the British Paediatric Association and was for some years a member of its council. He was a member and chairman of the Paediatric

Advisors' Committee of the South East Thames Region and got all of us to pull together and to plan in common for the future.

David continued to become involved in difficult and necessary causes: bereavement in children and their families and the need for paediatricians to become increasingly aware of the emotional aspects of bereavement. He produced with colleagues the report of the Royal College of Obstetrics Working Party on the management of perinatal death and participated actively in writing the booklet issued by the Stillbirth and Neonatal Death Society indicating those actions that should be followed on the death of a baby. This has provided a great deal of comfort to parents and indeed to the professionals who looked after them. He wrote and lectured extensively on this subject and on related issues such as the need to learn how to break bad news and to do so with warmth and affection. Not only gifted with exceptional ability to talk to people he was not afraid of showing his own feelings and did not restrain himself from physical contact when the occasion required the extra warmth and comfort that this would give. David I am sure lived by the precept that he set out in a beautifully written article on the bereaved child 'How Can We Help' and I would like to quote the last paragraph of this article:

'Children are honest and realistic and brave, this is what makes them so immensely attractive. If they are helped to face and recognise death, and, as far as possible, to understand their own feelings, and the very unhappy feelings of those around them, they can be spared some of the misery of unexpressed guilt, fear and confusion. And both they and we can learn how important it is to not to shrink from those who are suffering'.

I am sure that this is a lesson that David would like us adults and children to take to heart at all times and it is particularly apt for us to do so today.

David was a man of boundless energy. He played tennis for 20 years every other day until quite recently. He certainly could be described as being young at heart. He seemed even in his last years to have a youthfulness that many young people would envy. He loved life and was enormous fun. His optimism and cheerfulness never wavered even though at times he was extremely ill. In spite of this, and even in his last few years, there were occasions when it was possible looking at him totally to forget that he was at all in danger. His intrepid cycling took him all over London and he would pop up in the most unexpected places. David was irreverent and effervescent and took great delight in laughing at a number of things—including himself on occasion.

He was puckish and provocative, never afraid to ask those questions that needed to be asked and he did not seem to mind what he said or where he said it, provided it was said in a good and just cause. I never found him to be waspish or hurtful and his comments were never destructive. Perhaps 'cheeky' was the most acerbic response that I ever heard him give to any tart comment directed against him. Everyone I have spoken to has used the same sort of adjectives to describe him including 'lovable', 'loyal', and 'charismatic'. He seemed to make all things possible and he had an ability to break the ice and to put people at ease. He always seemed to me to be delighted when something good was being done.

His manner and behaviour were courteous, though slightly disrespectful when pomposity needed to be put in its place. He had a generosity of spirit and unparalleled optimism. He had a great concern for the underdog. His vigil outside South Africa House, which was prompted by his daughter's involvement in the anti-apartheid movement, was fundamentally a demonstration of his deep concern for the welfare of the down trodden and this conversely was one area where he showed that any anger that he possessed was reserved for the tyrannical and cruel.

We extend our deep sympathies to his wife and family. They will all miss him but I feel sure that they will remember his full and fine life. I hope too that they will know that their loss is shared by us, and will have to be borne by the countless children and parents whom David cared for, the organisations he founded and nurtured, and the friends he captivated. All of us remember the twinkle in his eye, the cheerfulness he generated, the work he started, the attitudes he changed permanently, and the kindness and consideration he tirelessly sought to extend to those most neglected. These will remain the permanent legacies of his benign spirit.

I would like to end with the last line from the address that was made not too long ago at Jacqueline Du Pré's funeral service. I feel sure that you would agree that this sentence is equally apt when considering the life of David Morris as it was on that sad occasion:

'Let it not be said that life was good to us but rather that we were good to life'.

I am sure that this will be said of David—a dearly loved man.

Archives of Disease in Childhood, 1989, **64,** 1513–1516

Correspondence

When to do a lumbar puncture in a neonate

Sir,

We enjoyed Dr Halliday's annotation on neonatal lumbar puncture but felt that his discussion of the role of lumbar puncture in suspected sepsis left an unfortunate question mark hanging over this procedure.[1]

It is important we feel to distinguish between early onset and late onset sepsis. In early onset sepsis the usual route of infection is by ascending infection that causes neonatal pneumonia, often clinically and radiologically indistinguishable from hyaline membrane disease, and septicaemia. Frequently babies with group B streptococcal pneumonia are septicaemic at birth,[2] and as meningitis occurs secondary to high level bacteraemia it is scarcely surprising that about 30% of babies with early onset group B streptococcal sepsis have meningitis.[3 4] Dr Halliday quotes Eldadah and colleagues' two year study,[5] which showed only five cases of group B streptococcal pneumonia and septicaemia, none of whom had meningitis, as a reason for not doing early lumbar punctures. Over a five year period (1984–9) with 5000 to 6000 live births per year we have seen seven cases of early onset meningitis (<48 hours old): four were due to group B streptococci, and one each to *Listeria monocytogenes, Escherichia coli,* and *Streptococcus mitis.* There were no clinical features to distinguish them from septicaemia without meningitis. In two of the seven cases blood cultures were negative. A positive lumbar puncture caused us to revise our antibiotic regime in two cases.

The situation with suspected late onset sepsis is superficially similar but the organisms are different. Over the same five year period, we have seen four cases of Gram negative bacillary meningitis (one pseudomonas, one klebsiella, one achromobacter, and one with both klebsiella and *E coli*). In all cases the blood cultures were positive, but a positive lumbar puncture altered our antibiotic regime.

It is sometimes argued that if the same antibiotic regime is used to treat late onset septicaemia alone as is used to treat meningitis, then one can rely on blood cultures without performing a lumbar puncture. However, about 10% of late onset meningitis occurs with negative blood cultures.[6] If, as is increasingly the case, antibiotics are stopped after two or three days in the face of negative blood cultures, the occasional case of meningitis will be missed and will progress. Although rare, such an outcome would be disastrous. In addition, we have seen two unsuspected cases of *Candida albicans* meningitis and four cases of enteroviral meningitis over the same five year period. It is important to diagnose the former for early treatment and the latter to prevent cross infection.

We certainly believe that lumbar puncture can lead to significant respiratory deterioration in babies with hyaline membrane disease, particularly those with pulmonary hypertension, and we defer the procedure in such babies. In general, however, we believe that for both early and late onset sepsis, it is important that a lumbar puncture, which is after all a 'biopsy' of cerebrospinal fluid giving instant results, be performed. This affects prognosis and directs antibiotic and supportive treatment appropriately.

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Dr Halliday comments:

I thank Drs Isaacs and Dobson for their comments on my annotation.[1] If a question mark hangs over the role of lumbar puncture in suspected sepsis then I am afraid that the doctors from Oxford have not removed it. Most paediatricians would agree that all babies with early and late onset sepsis should have a lumbar puncture performed, but the problem is in making a diagnosis of suspected sepsis. The Oxford data do not help in this respect, showing a low incidence of early onset meningitis with only seven cases over a five year period. Drs Isaacs and Dobson do not state their indications for doing a lumbar puncture in suspected early onset sepsis but I suspect that many hundreds of babies had this procedure performed to pick up just two babies with meningitis whose blood cultures were negative.

The incidence of late onset meningitis in Oxford is also low with only four cases detected over five years—all of whom had positive blood cultures. While agreeing that about 10% of late onset meningitis occurs with negative blood cultures[6 7] this was not so in Oxford so that Drs Isaacs and Dobson would not have stopped antibiotics 'after two or three days in the face of negative blood cultures'. Surely it is unlikely that any paediatrician would stop antibiotics after two or three days in an ill baby who has negative blood cultures?

The picture remains unclear; we know little of the cost benefit ratio of neonatal lumbar puncture for many indications. Neonatologists must be sure that the procedures they undertake to screen selectively populations of neonates are not more hazardous overall than the disease that they are trying to detect.

Perhaps the only way to answer this question is to perform a randomised controlled trial comparing liberal use of lumbar puncture with more restricted indications as suggested in the annotation. Such a trial would have to be multicentre and look carefully at both short and long term outcome. Clinicians who are sure that their own practice is better would of course be excluded from such a study. Until firm evidence is available precise indications for lumbar puncture in the newborn will remain subjective.

References

[1] Halliday HL. When to do a lumbar puncture in a neonate. *Arch Dis Child* 1989;**64:**313–6.

[2] Pyati PSP, Pilades RS, Jacobs NM, *et al.* Penicillin in infants weighing two kilograms or less with early-onset group B streptococcal disease. *N Engl J Med* 1983;**308:**1383–8.

[3] Anthony BF, Okada DM. The emergence of group B streptococci in infections of the newborn infant. *Am Rev Med* 1977;**28:** 355–69.

[4] Baker CJ. Group B streptococcal infections. *Adv Intern Med* 1980;**25:**475–501.

[5] Eldadah M, Frenkel LD, Hiatt IM, Hegyi T. Evaluation of routine lumbar punctures in newborn infants with respiratory distress syndrome. *Pediatr Infect Dis J* 1987;**6:**243–5.

[6] Visser VE, Hall RT. Lumbar puncture in the evaluation of suspected neonatal sepsis. *J Pediatr* 1980;**96:**1063–7.

[7] Bell AH, Brown D, Halliday HL, McClure G, Reid MMcC. Meningitis in the newborn—a 14 year review. *Arch Dis Child* 1989;**64:**873–4.

Diagnosis of neonatal bacteraemia

Sir,

I would like to comment on two letters in your March 1989 issue. Miceli Sopo *et al* believe that values for positive predictive accuracy of '56·8%, 59%, or 39–62% are unacceptable' while pointing to the 'great potential usefulness of the negative predictive accuracy of the evaluated tests'.[1] However, they neglect to mention the incidence of sepsis in the populations evaluated. As my paper was quoted,[2] I should indicate that the incidence of sepsis in that study was only 8%, so that a positive predictive accuracy of 39% has improved predictiveness by almost five times—hardly 'unacceptable'. Conversely, the chance that sepsis was not present was 92%, so that the negative predictive accuracy of 99% is not quite as impressive as it might at first appear.

In their study there were 17 infected babies (15 with documented sepsis) among 70 preterm infants, with positive predictive accuracy of 59% and negative predictive accuracy of 91·6%.[1] These numbers should be compared with the incidence of infection of 24% and 'no infection' of 76% (that is, the chance of predicting each category without the tests being available).

Dellagrammaticas *et al* report their experience with C reactive protein,[3] which suggests that the response may vary with the infecting organism, that sensitivity is lower in the first 24 hours after delivery, and that C reactive protein needs to be considered together with haematological tests. Like Mathers and Pohlandt,[4] I have observed that C reactive protein may lag behind the immature/total neutrophil quotient, especially in early onset infection due to group B streptococcus.[5]

These observations emphasise the difficulty in evaluating any diagnostic tests for sepsis, because different organisms may produce different patterns of response. The paper by Kite *et al* was heavily weighted with cases of coagulase negative staphylococcal bacteraemia (21 of 34).[6] The lack of sensitivity of the immature/total neutrophil quotient with this organism has previously been noted,[7] whereas it is more likely to be deranged with early onset group B streptococcal infection.[4 5]

It is difficult to escape the conclusion that we need to evaluate more than one test when attempting to diagnose neonatal bacteraemia and that different organisms (as well as different severity of illness) may produce different responses.

References

[1] Miceli Sopo S, Pesaresi MA, Pastore M, Stabile A. Comparison of five tests used in diagnosis of neonatal bacteraemia. *Arch Dis Child* 1989;**64:**428.

[2] Philip AGS, Hewitt JR. Early diagnosis of neonatal sepsis. *Pediatrics* 1980;**65:**1036–41.

[3] Dellagrammaticas HD, Tapratzi T, Kourakis G. Comparison of five tests used in diagnosis of neonatal bacteraemia. *Arch Dis Child* 1989;**64:**429.

[4] Mathers NJ, Pohlandt F. Diagnostic audit of C-reactive protein in neonatal infection. *Eur J Pediatr* 1987;**146:**147–51.

[5] Philip AGS. Response of C-reactive protein in neonatal group B streptococcal infection. *Pediatr Infect Dis J* 1985;**4:**145–8.

[6] Kite P, Millar MR, Gorham P, Congdon P. Comparison of five tests used in diagnosis of neonatal bacteraemia. *Arch Dis Child* 1988;**63:**639–43.

[7] Schmidt BK, Kirpalani HM, Corey M, Low DE, Philip AGS, Ford-Jones EL. Coagulase-negative staphylocci as true pathogens in newborn infants: a cohort study. *Pediatr Infect Dis J* 1987;**6:** 1026–30.

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Does dexamethasone suppress the ACTH response in preterm babies?

Sir,

We are interested in the need to consider steroid replacement treatment for intercurrent infection or surgery in babies who had been on dexamethasone for chronic lung disease. We used a protocol similar to that of Rennie *et al* for dexamethasone and stimulation by adrenocorticotrophic hormone (ACTH). The cortisol responses to tetracosactrin (Synacthen, Ciba) in our six babies were similar to those reported in their paper.[1] The tetracosactrin (36 μg/kg) was given to six infants one week after the dexamethasone had been stopped and again four weeks later to four infants; the results are shown in the table.

We would agree with Rennie *et al* that steroid replacement treatment may not be necessary. In our series all of

Table *Cortisol concentrations after treatment with tetracosactrin. Results are median (range)*

	One week after stopping dexamethasone (n=6)	*After further four weeks (n=4)*
Cortisol concentration (nmol/l):		
Baseline	128·3 (25–400)	276·0 (30–375)
After tetracosactrin	802·5 (345–1420)	1065·0 (380–1560)

the babies responded to tetracosactrin even if their baseline concentrations of cortisol were low. By contrast three babies out of eight tested by Arnold *et al* with low baseline concentrations of cortisol did not respond appropriately at initial testing but had normal responses one month later.[2] We would presume that babies with ongoing problems are less likely to respond normally and that in such infants baseline cortisol concentrations should be assessed at times of additional stress, at least for the month or so after cessation of dexamethasone. Babies without ongoing problems appear likely to have normal responses and baseline cortisol concentrations may be expected to be normal.

References

[1] Rennie JM, Baker B, Lucas A. Does dexamethasone supress the ACTH response in preterm babies? *Arch Dis Child* 1989;**64**:612–3.

[2] Arnold JD, Leslie GI, Williams G, Rack P, Silink M. Adrenocortical responsiveness in neonates weaned from the ventilator with dexamethasone. *Aust Paediatr J* 1987;**23**:227–9.

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Immunoreactive trypsin in Shwachman's syndrome

Sir,

Dr Dossetor and colleagues report finding low serum immunoreactive trypsin in two children with Shwachman's syndrome.[1] Their observation, however, together with the suggestion that a low serum immunoreactive trypsin may obviate the need for invasive tests of pancreatic exocrine function, hardly seems novel. Low serum immunoreactive trypsin(ogen) concentration has previously been described in Shwachman's syndrome and low values are known to correlate with low output of trypsin in response to stimulation testing of the pancreas.[2] [3] As cystic fibrosis is the only cause of pancreatic insufficiency more common than Shwachman's syndrome in young children and is associated with a raised serum immunoreactive trypsin, it is evident that a low serum concentration is likely to point to the diagnosis of Shwachman's syndrome. Through screening large numbers of children the authors have shown that a low serum immunoreactive trypsin as a test for pancreatic exocrine insufficiency has a high specificity, but apart from this, isn't this report more a case of reinventing the wheel?

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Drs Dossetor and Heeley comment:

The initial title of our paper was 'Immunoreactive trypsin in Shwachman's syndrome in early infancy', but this was shortened in revision for publication. Unfortunately this has resulted in Dr Puntis missing the point of our paper, which was to show the value of the immunoreactive trypsin test in the investigation of an infant with malabsorption. In the paper of Durie *et al*,[2] the age of the patients is 2·25 to 18 years and in that of Moore *et al*,[3] the mean age of the patients is 5·9 years (although in this paper there may have been one infant with Shwachman's syndrome, but it is not made clear).

Also a serious flaw in these two publications lies in their control values. In the 1981 paper the mean immunoreactive trypsin in controls under 2 years is 7 μg/l and over 3 years 13 μg/l[2]; but in the 1986 paper, the mean immunoreactive trypsin in controls has arisen to 31·4 μg/l with no change in the methodology.[3] In patients with pancreatic steatorrheoa, a mean value of 4·9 μg/l is found, so that certainly the authors show low values in pancreatic disease. We, however, showed undetectable concentrations in two infants with Shwachman's syndrome against a larger number of controls at different ages of infancy, establishing beyond doubt the diagnostic value of the test.

We feel the value of the test is insufficiently known to general paediatricians. With the spread of screening for cystic fibrosis, the immunoreactive trypsin test is now generally available. The diagnosis of significant pancreatic acinar deficiency is as easy as diagnosing iron deficiency with this test. We could, however, agree with Dr Puntis on one point, that in the investigation of an infant with malabsorption, the immunoreactive trypsin test ranks in importance with the discovery of the wheel.

References

[1] Dossetor JFB, Spratt HC, Rolles CJ, Chan Seem CP, Heeley AF. Immunoreactive trypsin in Shwachman's syndrome. *Arch Dis Child* 1989;**64**:395–6.

[2] Durie PR, Largman C, Brodrick JW, *et al.* Plasma immunoreactive trypsinogen in cystic fibrosis: a sensitive indicator of exocrine pancreatic dysfunction. *Pediatr Res* 1981;**15**:1351–5.

[3] Moore DJ, Forstner GG, Largman C, Cleghorn GJ, Wong SS, Durie PR. Serum immunoreactive cationic trypsinogen: a useful indicator of severe exocrine dysfunction in the paediatric patient without cystic fibrosis. *Gut* 1986;**27**:1362–8.

The thermal environment in which 3–4 month old infants sleep at home

Sir,

Dr Wailoo and colleagues have provided valuable data in an area that has hitherto received little attention but may be of considerable clinical relevance.[1] It would be helpful to have more information on two points. First, in calculating the total insulation of clothing and bedding did they make allowance for the proportion of the baby covered by each item? For example, a cardigan and a duvet will normally cover about ⅓ and ⅘ of the baby's surface area: if the tog values of the materials are 2 and 10 the contributions to total insulation will be about 0·7 and 8 tog respectively. If no such allowance was made their totals will be overestimates. Second, did they take into account babies' tendency to throw off bedding at night, which may considerably reduce their insulation?

As the authors suggest, comparison of a baby's insulation in bed at night (that is, clothing plus bedding) with insulation before he goes to bed (that is, clothing only) is not really meaningful because while awake and active his metabolic rate may be higher; a more valid comparison would be with insulation used for sleeping in the day.

Finally, it should be noted that the formula of Burton and Edholm that they quote relates to clothing in adults,[2] and its applicability to the bedding and clothing of babies is uncertain.

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Drs Wailoo and Petersen comment:

We are grateful to Dr Bacon for giving us an opportunity to clarify aspects of our paper that we have considered more fully since it was submitted last year.

We are also concerned about the consequences of babies being unevenly and incompletely covered by clothing and wrapping, but allowance cannot be made in the way Dr Bacon suggests. 'Tog value' refers to the insulating effect (standardised to 1 m^2) of fabrics, not garments. A total tog can only be calculated if all fabrics cover the same area, and this reflects total thermal insulation on a body only if it is completely covered, and allowance made for its actual area. It is not accurate to treat a fabric covering part of a body as though it were one of lower insulation covering the whole. Areas of low thermal resistance will shunt heat around those that are higher, and where parts are uncovered the heat flow through them may dominate the total. A fabric of 20 tog over 10% of the body will be very much less insulating than 2 tog fabric over the whole.

The accurate determination of heat flows from various parts of an unevenly insulated body of varying surface temperature is a complex problem, but we can simplify the situation for a baby in its cot by considering it as two parts, a well insulated body under wrappings, and poorly insulated exposed parts such as the head. The figure we quote in the paper is the 'tog value' of the insulation on the *covered parts*, assuming garments such as nappies and vests to be evenly spread under the blankets, which will introduce a small, but tolerable error.

This figure is very useful to examine parental behaviour, but, as we indicate in our discussion, assessing its impact on heat balance is another problem, which we are still considering. Our measurements of surface temperature indicate that 70–90% of the sleeping baby's body heat is lost by the head and other exposed parts. Changing insulation on the body, unless it becomes very low, does not much affect heat loss, but factors influencing heat loss from the head, such as ambient temperature, are critical.[3] In this context, although we did not observe babies throwing off their wrappings, they did often expose their hands, which will in fact have a much greater effect on heat balance.

The formula of Burton and Edholm, which we quote merely for comparison, was modified by Clulow for a baby's metabolic rate and body shape, but did assume a fixed 40% of heat loss from the head, which we now consider too simple. We are working on a more suitable model.

We are now able to report mean (SD) data on wrapping during daytime sleeps: babies are covered with 12·1 (0·9) tog in rooms at 19·5 (1·2)°C, though it is not clear to us whether comparisons between different conditions are useful for anything other than illustration.

We are continuing our studies, and will be able to report more fully on thermal balance in the cot very soon.

References

[1] Wailoo MP, Petersen SA, Whittaker H, Goodenough P. The thermal environment in which 3–4 month old infants sleep at home. *Arch Dis Child* 1989;**64:**600–4.

[2] Burton AC, Edholm OG. *Man in a cold environment.* New York: Edward Arnold, 1955.

[3] Anderson E, Wailoo MP, Petersen SA. Keeping babies warm—have we got it right? *Health Visitor* (in press).

Archives of Disease in Childhood, 1989, **64**, 1517–1525

Autumn books

My favourite textbooks

Libraries and bookshops create a mixture of peace and panic in me. Peaceful surroundings, a break from the bustle of the week, the immobility of books, soft voices: then open a book and feel the drive to read for information not yet known or remembered, ideas not yet encountered. Oh for more time to read; all too soon the free half hour is over. Back to the bustle of work, with an ever increasing 'information anxiety state'.

To be a good doctor is, at least in part, to have good knowledge. Textbooks need to impart knowledge at every level, from first contact to specialist revision and reference. Fortunately medicine is not all knowledge and therefore an approach to the practice of medicine in terms of doctor-patient relationships and other concepts is needed. Speed and interest are essential especially at the first contact level and later for revision; the practice of paediatrics and availability of free time being mutually exclusive.

I did manage to read Levene's *Essentials of Neonatal Medicine* in two hours, admittedly with some neonatal knowledge already. *Paediatrics* by Barnes and Roberton (Update Books) immediately gave a feel for the subject being well illustrated with photographs, growth charts, and summary lists, and being short enough to read fully in a couple of sittings. *Neonatal Medicine* by ML Chiswick had a similar appeal, but of course, is now out of date.

Essential Paediatrics by David Hull and Derek Johnston is too long on didacticism and too short on problem solving for me, and yet numerous medical students still find this a good compromise. Recently MJ Robinson has written *Practical Paediatrics* and combined a symptom or problem orientated approach with a sound basic coverage of paediatrics to produce the ideal student's textbook, hampered only by its Australian figures and a different definition of perinatal to the usual.

'Doctor, can you please come and see this child with . . .', often causes *Aids to Paediatrics* to surface and in 30 seconds the mind has some new ideas to debate on the walk over to the ward. *Aids to Postgraduate Medicine* also jogs the memory and is used repeatedly in revision for the MRCP examination. Illingworth's *Common Symptoms of Disease in Childhood* would be nice to have but was financially hard to justify in addition.

As a senior house officer and junior registrar I was never separated from my *Paediatric Vade Mecum*, Roberton's pocket sized *Manual of Intensive Care*, and the *Alder Hey Book of Paediatric Prescribing*. I bought Nelson's *Textbook of Pediatrics* cheaply and have read three to four pages of it but really it does more for the panic than the peace in me. I refer to the *Textbook of Paediatrics* by Forfar and Arneil on the rare occasions that I have had time to. It needs to be read at a desk, not in a lift. For reference I do need access to *Neurologically Handicapped Children: Treatment and Management* by Neil Gordon and Ian McKinley; *Recognisable Patterns of Human Malformation* by Smith; *Clinical Paediatric Nephrology* by RJ Postlethwaite and *Essential Medical Genetics* by JM Connor and Ferguson Smith. I first learned that sunsetting can be normal, as can eight loose stools a day, by reading Illingworth's *The Normal Child*—one of the few textbooks read from cover to cover. To maintain a love and fascination for children I will read and reread *Mr God, this is Anna* by Fynn, *Dibs in Search of Self* by Virginia Axline, and Sula Wolf's *Children in Danger*.

As I have grown up in paediatrics and acquired more review articles, these are often used now for the latest state of the art finds. I know that textbooks are often out of date by the time of going to press but somehow the physical presence of Roberton's *Textbook of Neonatology* maintains more air of authority than flimsy blurred photocopied articles. Its very permanence tends to instil calm as one notes with relief that it does still say the same thing as the last time it was read.

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My early childhood love of reading, and my marriage into a family of writers, has nurtured my enthusiasm for books. Most junior doctors, however, having survived undergraduate examinations, fail to associate reading with pleasure. Any textbook must look attractive and have clarity and brevity of text to alert the prospective reader. *Hospital Paediatrics* by T Milner and D Hull is such a book, although I consider it to be vastly overpriced in its paperback form and its texts on management of conditions are sometimes too brief.

Moving onto the larger textbooks, the old favourites are *Textbook of Paediatrics* by JO Forfar and GC Arneil and Nelson's *Textbook of Pediatrics* by RE Behrman, VC Vaughan, and WE Nelson. One feels duty bound to wade through them for paediatric MRCP. There is a limit, however, to the amount of stimulus any junior doctor's neurones can take as a consequence of being on call—it is all too easy to drift into the 'land of nod' in the hospital library. One has to be selective when using these volumes and there is often a lack of up to date information on investigations and management, as most hospital libraries do not stock the most recent editions.

Whether one wears a white coat or not, we have all carried pocket sized books next to our skin to calm the nerves when faced with diagnostic dilemmas. From personal experience, I have found the *Paediatric Vade*

Mecum, Alder Hey Book of Paediatric Prescribing, Aids to Paediatrics by A Habel and the paperback bible of neonatology, *A Manual of Neonatal Intensive Care,* invaluable companions.

Having cleared the hurdles of postgraduate examination the middle grade paediatrician is faced with teaching the reluctant undergraduate. This art can only succeed if it promotes easy recall of information, prevents sleep, and creates an impetus to use library facilities. Consequently, I have based my lectures on information from the aforementioned books, personal experience, but mainly from annotations and reviews in journals, such as the *Archives of Disease in Childhood, British Medical Journal, The Lancet,* and *Hospital Update.*

Lastly, I am indebted to Professor Ronald Illingworth for sharing his wealth of paediatric knowledge through writing. All paediatricians should read *The Normal Child* to appreciate the wide range of body function, whether it be stool frequency or sleep patterns. I have often consulted the *Development of the Infant and Young Child,* with its delightful illustrations. *Symptoms and Signs of Disease in Childhood* emphasises the importance of history taking and clinical examination, often overlooked in these high tech days. My wholehearted thanks to Professor Illingworth for guiding me through my paediatric training.

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Manual on Infections and Immunizations in Children. Edited by Angus Nicoll, Peter Rudd. Pp 349: £9·95 paperback. Oxford Medical Publications, 1989. ISBN 0-19-261785-0.

Immunisation against Infectious Disease. Joint Committee on Vaccination and Immunisation. London: HMSO, 1988. (The Green Book.)

There is considerably more to the manual than the foreword by Edwina Currie, 'a Minister with a commitment to prevention and health promotion'. It is loosely modelled on the 'Red Book' on paediatric infections produced by the American Academy of Pediatrics and updated every two years. Primary paediatric care in the United States is largely provided by primary care paediatricians and the Red Book is almost a bible: you can deviate from the scriptures but at your peril and likely your patient's peril too.

Is the BPA manual aimed primarily at general practitioners, hospital or community based paediatricians, or does it have a potentially wider audience including clinical medical officers, health visitors, and medical students? I would say that all the above would find value in this book, which might be construed as a polite way of saying that the target readership was somewhat unclear.

The manual opens with interesting epidemiological data on incidence and mortality of childhood infections. The first main section deals with clinical problems (neonatal infection, respiratory infection, rash, fever, urinary tract infection). This section could readily be expanded, for the benefit of junior and senior hospital paediatricians, to include other clinical scenarios (for example, infections of bone, soft tissue, and the central nervous system).

The second section covers infections caused by almost 50 specific pathogens. This is the uneasiest part of the manual. In the interests of brevity the topics are often covered too cursorily to do more than drive the reader to a more definitive text. Some annotations, such as that on HIV infection, are valuable summaries but others (all *Haemophilus influenzae* infections in two pages) are too condensed. My main concern about this section again was its target audience.

The third section covers immunisation and closely follows the excellent Department of Health publication known as the 'Green Book' The influence of Dr David Salisbury, an ex-paediatrician, and Dr Christine Miller from Colindale can be clearly seen in the Green Book, which finally makes some sense of the indications and contra-indications for childhood immunisations. The BPA Steering Committee responsible for the manual wisely asked Dr Salisbury to sit on the committee and there is strong consistency between the manual and the Green Book. There is little fence sitting now in either book on important issues. The contraindications to pertussis immunisation are clearly delineated and, although some are less controversial than others (why is fever to 39·5°C within 48 hours of pertussis vaccine a contraindication to further doses?), the authors are to be congratulated on a difficult job well done.

The fourth section of the manual deals with practical issues surrounding immunisation, the sort of every
problems such as egg allergy and dela
immunisations on which Dr Nicoll
many others are frequently consult
These are beautifully handled,
together with the introduction to the th
section covering general points on vaccin
consent, and technique provide a valua
adjunct to the Green Book, of use
'givers' and 'advisers'. One carp:
p 258 the authors state that immu
depressed children should not be given
vaccines, yet later on the same page t
immunodepressed children *must*
immunised with the mumps, measl
rubella vaccine (the former is corr
except for children with HIV infectio
This unfortunate apparent contraindicati
is due to poor wording and should
corrected for the next edition.

The bane of my life is enquiries ab
malaria prophylaxis so I am extrem
grateful to the authors for the fifth secti
on travel abroad, and particularly
antimalaria recommendations, which
difficult to find elsewhere. These are
current recommendations from
London School of Hygiene and Tropi
Medicine, soon to be changed, so althou
the rationale for the different regimes
incomprehensible, at least we can be c
sistent.

The manual is therefore something o
curate's egg (no reference to Edwi
intended). The good parts are often v
good, and the first two sections could
expanded or modified in what I hope
be regular revisions of this valuable boo
think the BPA are to be applauded
their brave initiative in undertaking t
venture, and hope they are encouraged

ake this the first of a series of BPA ndbooks.

would like to acknowledge Dr Martin Moncrieff's sistance in reading the book and providing lpful comments.

D Isaacs
WELLCOME TRUST LECTURER
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nmunization in Practice. World Health rganization. Pp 361: £3·50 paperback. xford University Press, 1989. ISBN -19-261545-9.

his book is an excellent example of a aching manual with its emphasis on articipation rather than lectures. It is clear nd simple without being patronising. I ow know how to sharpen needles, store ater for reuse, and to put the box of accine in the shade of a tree, if my car reaks down.

The WHO gives immunisation a high riority. Training large numbers of health orkers to administer vaccines is vital and ey have drawn up a training programme or these people. This programme has been sed, modified, and rewritten. They have ow published this as a training manual. It primarily for workers in developing ountries, but I think its approach to aining and practical ideas have much to ffer people in 'developed' countries. How ften do we forget the importance of the dvice 'be reliable and punctual', 'be polite nd friendly'?

The opening pages discuss approaches to aining. There is an emphasis on helping eople to understand by means of exercises, uestions, case studies, and discussions, ather than lectures. There is also specific dvice on planning a training session. The est of the book is divided into eight odules for field workers on practical spects of running an immunisation rogramme—for example, vaccines and ow to look after them, preparing for an nmunisation session, how to evaluate our own immunisation programme. Each odule consists of clear, simple information ith appropriate practical work. Widepread use of the programme should have oned out most problems. The error in belling the illustrated syringe (0·5 instead f 0·05) is presumably because in practice a eal syringe is used for demonstration urposes.

This material would be essential for nyone working in the field of immunisation in developing countries. The original modules have apparently not always been easily obtainable. The book gives a valuable insight into the problems faced by immunisers in developing countries—for example, the emphasis on maintaining a cold chain and the practicalities of organising outreach sessions in the open air. It also illustrates the positive attitude to immunisation by the WHO (children should be immunised even if unwell). It is also a very good model of teaching methods applicable in all countries in many fields.

C Pullan
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Diseases of the Fetus and Newborn: Pathology, Radiology and Genetics. Edited by G B Reed, A E Claireaux, and A D Bain. Pp 812: £135 hardback. Chapman and Hall, 1989. ISBN 0-412-27990-8.

This book attempts to mould into one volume information for the pathologist, radiologist, and geneticist. The text is in four sections: (1) general aspects of disease, (2) systemic pathology, (3) radiology and imaging, and (4) medical genetics. In the preface the editors say that their targets are the pathologist, laboratory scientist, obstetrician, and paediatrician.

To reach such a wide audience they have recruited 59 contributors from six countries, including 29 from the United States and 20 from the United Kingdom. Editorial control of this number of contributors must be extremely difficult and American and English spellings are used in various chapters so that one reads about 'paediatrician' and 'pediatrician' and 'foetus' and 'fetus' depending upon the origin of the author. Another problem faced by the editors was delayed publication. On page 637, Drs Simoni and Brambati from Milan state at the end of their chapter on fetal karyotyping: 'In December 1985, when we wrote this chapter, we focused the technical and diagnostic problems related to first trimester CVS on the basis of our experience that was just lower than 1,000 cases. By now (June 1988) our experience has been extended to 2520 diagnoses . . .'. Contributors who submit their chapters early have more work in updating but the Italian doctors managed to include six references from 1986 and two from 1987 in their updated chapter. The death of Dr Douglas Bain, one of the coeditors, in January 1987 must have been a major reason for delay in publication.

Overall this is a book for the pathologist rather than the clinician. Of the contributors there are only one or two obstetricians and paediatricians but no neonatologist. The longest section, on systemic pathology, is probably the best, although there are some areas of overlap. The section on radiology is quite short but is liberally illustrated with radiographs, ultrasound scans, and magnetic resonance images.

The fourth section, on medical genetics and prenatal diagnosis, begins with a scholarly discussion of genetic screening by Dr Seamus Cahalane from Dublin. He begins by quoting from Shakespeare's *Love's Labour's Lost:* 'These are begot in the ventricle of memory, nourished in the womb of pia mater, and delivered upon the mellowing of occasion'. These words, although uttered as pretentious jibberish by the pedant are as apt a description of the chapter as is the irony of the play's title. Later in this section Drs Alan Michelson and Stuart Orkin from Boston provide a concise summary of prenatal diagnosis by analysis of fetal DNA. Gene mapping, DNA cloning, southern blotting, and restriction fragment length polymorphisms all crop up as expected in modern textbooks. but they have a role in prenatal diagnosis not only of haemoglobinopathies but also of phenylketonuria, ornithine transcarbamylase deficiency, Duchenne muscular dystrophy, and cystic fibrosis.

The editors have been largely successful in their aim to produce a tripartite textbook. Future editions would be improved by correction of areas of overlap. This book should be most useful to practising and training perinatal and general pathologists and a copy should be available in all pathology departments. It will also provide a useful reference for practising obstetricians and paediatricians and each large maternity hospital should have one. At a price of £135 I doubt whether many paediatricians will buy a personal copy.

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Practical Genetic Counselling. By Peter S Harper. 3rd ed. Pp 306: £25 paperback. Butterworth, 1988. ISBN 0-7236-1019-3.

A 'simple book' is offered by the author, yet he provides a discursive exposition of the whole subject from basic principles to details of latest DNA technology. He succeeds in appealing to his intended audience of practising clinicians. The first 10 chapters are general, covering mendelian and non-mendelian disorders, chromosome anomalies, dysmorphology, carrier detection, prenatal diagnosis and the organisation of a genetic counselling clinic. The section on X linked disorders conveys its complexity and will convince readers of the necessity for clinical geneticists!

The second half of the book covers 'specific organ systems' with chapters which include oral and craniofacial disorders, deafness, environmental hazards, genetic risks in cancer, in addition to predictable topics. Here there is a tendency to repeat some information from the early chapters, and much space is given to clinical description rather than genetics per se. Many non-genetic conditions are briefly included. There is a comprehensive index and a list of lay societies involved in inheritable diseases. There are some excellent tables but these could have been used much more extensively in the organ system section where many lines of text convey little information and abbreviations are not much employed.

The style is fluent and persuasive. Professor Harper displays clear empathy with his audience, shining through as a physician whose appreciation of the human aspects of his subject is paramount and to which his knowledge is subjugated. Anecdotes, imperatives, and aphorisms appear regularly, rendering the reading most enjoyable and inviting us to match both his enthusiasm and professional rigour. His concluding chapter touches on eugenics and looks to the future. The book is practical, informative, and stimulating—highly recommended.

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Clinical Paediatric Endocrinology. 2nd ed. Edited by CGD Brook (forward by MM Grumbach). Pp 724: £125 hardback. Blackwell Scientific Publications, 1989. ISBN 0-632-02018-X.

'The dramatic advances in endocrinology . . . form . . . an extraordinary chapter in the history of science and medicine. The profound effect . . . on paediatric endocrinology . . . is of special interest' to paediatricians. The words are those of Dr Mel Grumbach who provides the forward to this comprehensive resource text book.

There are 31 contributing authors, 19 based in the United Kingdom and the remainder originating from Europe or the United States reflecting the editor's affiliations with the European Society for Paediatric Endocrinology and the Lawson Wilkins Society.

A number of chapters by the same authors as the 1981 edition have required little revision but others are comprehensively updated and retain their excellence, such as chapters on intersex (MO Savage), the short child (JM Parkin), the neurohypophysis (J Perheentupa), adrenal deficiency and excess (M Forest). Several chapters with similar subject headings to the first edition are written by different authors, for instance the first chapter on fetal growth (DJ Hill and RDG Milner) provides a fascinating review of paracrinology, the tissue signals responsible for early embryological growth and later endocrine development.

The Middlesex approach to investigation, description, and management of short stature is thoroughly aired in chapters on normal growth and endocrine control and the management of short stature. Many readers hoping for practical advice will find these parts of the text somewhat theoretical, unduly mathematical, and at times unconvincing. The Middlesex authors redeem themselves with excellent accounts of puberty and its disorders.

Other slight disappointments were the chapters on gynaecology (lack of paediatric orientation with inappropriate therapeutics) and the thyroid gland (no mention of the consequences of late treatment of congenital hypothyroidism or the spectacularly improved prognosis after neonatal screening).

Further on in the book, however, there are useful chapters on hypothalamic hormones (A Grossman), the adrenal cortex (J Honour), and adrenal hyperplasia (M New and PW Speiser). There is an outstanding account of calcium disorders (K Kruse) and much sound scholarship and practical advice on diabetes mellitus (B Weber and F Bonnici).

The entirely new section on imaging (computed tomography, nuclear magnetic resonance, and ultrasound) is successful except that several of the anatomical descriptions and figures would have benefited from additional line diagrams, arrows, and landmarks. Normal laboratory values and endocrine function tests (IA Hughes) are available in abundance.

This is, therefore, a provocatively different second edition which complements the first. It is for paediatricians and endocrinologists but they might be surprised by a lack of print quality and many typographical errors in a book of such horrendous price. It is disheartening that 'Next produces its 'directory' of 474 superbly printed colour pages for sale at £3 when it costs individuals or libraries £125 to buy this important text book.

P G F Swif
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Handbook of Paediatrics. By N Mann and A Nicoll. Pp 356: £12·50 paperback Blackwell Scientific Publications, 1989 ISBN 0-632-01967-0.

This book will be very popular for all those at the sharp end of paediatrics, whethe they are working in hospital, the community or general practice. There is an unusual bu wise choice of subjects, described in a fairly didactic way, and these cover most of the common and often difficult decisions tha have to be made.

As might be expected from these author there is fairly detailed discussion on growth development, and immunisation including good advice on AIDS. The accompanying charts and diagrams are well selected and highly relevant. Those with a passion fo flow charts will not be disappointed, the excellent one on resuscitation of the new born will no doubt be in the pocket of mos neonatal housemen, unless already taken from the authors' original article. Th more convoluted flowchart on the collapsed child is harder work but contains a mass o information.

The chapter on nutrition and fluids i particularly clear and easy access to all th usual formulae will be welcomed. Chil abuse is dealt with in a wise way, goin beyond the usual description of injuries giving good advice on case conferences reports, procedures, and courts.

The chapter on practical procedures i clearly written by someone still at th height of his technical skills and will b invaluable to all new housemen.

It is good to see brief but informativ chapters on death, paramedical services and immigrant groups.

This book is more than a vade mecur but would not see someone throug bership. It is essentially practical and uch fills a gap and will be much eciated by paediatric staff and general itioners.

P R H Barbor
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University Hospital, Nottingham

British Paediatric Association 1928–. By J O Forfar, A D M Jackson, and Laurence. Pp 228, hardback. British liatric Association, 1989. (Copies can btained from the BPA, price £5.)

authors deserve sincere congratulations he enormous amount of work that they put in to the preparation of what is, for many generations will remain, a rical document about the origins, , considerable achievements, and s for the further development of the sh Paediatric Association.

e book is an impressively compre- ive factual account of the BPA, from rigin in 1928 when six doctors met to ss its foundation, to 1988, when there ver 2000 members. It is illustrated and ened by 54 photographs of members, other illustrations, and by 78 profiles biographies of past and present bers.

bjects discussed include the organisa- of the BPA, the work of the Council academic board, the surveillance unit, numerous specialist groups, and the y diverse committees on all aspects of l health at home and overseas.

ther subjects include accounts of ious annual scientific meetings dermere, Scarborough, Aviemore, aster, Harrogate, and York), the lishment of the Diploma in Child lth and the *Archives of Disease in dhood*, and the various medals and rds, including the valuable Heinz owship for doctors in developing tries.

ere are extensive sections on the d for integration of the child health ices (Court and Brotherston reports), on the thorny question of the future of BPA in relation to the Royal Colleges, aps as a College or Faculty of Paedia- .

he book is a major and important ribution to the whole subject of iatrics.

R S Illingworth
Sheffield

Interstitial Lung Diseases in Children. Vol I, II, and III. Edited by Lourdes R Laraya-Cuasay, Walter T Hughes. Pp 583: £221·50 hardback. CRC Press Inc, 1988. ISBN 0-8493-4300-3.

Three slim volumes successfully review many respiratory diseases where standard texts provide little more than a paragraph or two. Forty three authors have written on a miscellany of topics where knowledge is still growing, as is evident by the conciseness of many chapters. This text therefore allows rapid reference for many of the more unusual respiratory conditions in childhood. It would be even more rapid if a single complete index appeared at the back of each volume. Lung diseases may not always be easily compartmentalised, for example, into either airway or interstitial disease. Thus differential diagnoses are often discussed within general chapters on pulmonary function, immunology, radiology, and the roles of lung biopsy and bronchoalveolar lavage.

There are 17 chapters covering the vast range of pathogens causing pneumonia or pneumonitis, or both, including pneumonitis in AIDS and bone marrow transplant recipients. Neonatal and viral pneumonias have relatively less prominence, but these are well covered in other textbooks. Other chapters include lung diseases caused by physical and chemical agents, drugs, lymphoproliferative and vascular disorders, renal, rheumatoid, neurocutaneous, and neoplastic diseases. Pulmonary sarcoid, haemosiderosis, and an array of other rarities complete the collection. All subjects are extensively referenced and numerous radiographs and histopathological slides, albeit in black and white, very adequately illustrate the text.

These books will be most suited to the paediatric respiratory centre, which will need to consider whether a reference text that may soon need to be updated is worth the price. However, I do not foresee a cheaper or British equivalent in the near future.

M Samuels
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Paediatric Radiology. By Christopher O'Callaghan. Pp 189: £19·50 hardback, Wolfe Medical Publications Ltd, 1989. ISBN 0-7234-0978-1.

I received this book initially with some mystification—what could I say about a paediatric radiology text? Looking more closely I discovered it to be compiled by a paediatrician and aimed at general paediatricians and radiologists. However, its format—illustrations accompanied by questions with answers and explanations overleaf—makes it clear that its chief market will be with candidates for membership. That stage in my life is mercifully over and leafing through the book served to remind me what a relief that was. On offering it to those of my colleagues still on the wrong side of the membership hurdle, however, they fell on it with ravening enthusiasm and passed it among themselves jealously. There is no doubt that it fills a yawning gap in the examination aids market. The comparatively recent establishment of 'paediatric part II' means that almost any paediatric self assessment text will be in demand, with preparation for the slide section in particularly short supply.

This book fills the gap admirably. The reproductions were generally good—though some chest films reproduced poorly. The range of subject is broad and appropriately general, avoiding the obscurities so beloved of examination candidates, but much less evident in the real examination and almost absent in clinical practice. It would therefore be churlish to complain that more rarified imaging is covered only briefly, with even ultrasound covered only thinly. As such, though very suitable as a basic postgraduate text, it would not really serve as a reference volume, despite a helpful index.

My colleagues so approved of this book that it was only with difficulty that I retrieved it to write this review. With over 200 illustrations the price is very reasonable, though it would still make a substantial dent in an individual's book budget. It would, however, be a modest purchase for any library and I would highly recommend it.

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PAEDIATRIC REGISTRAR
Guy's Hospital, London

The Embryology of Behavior. By Arnold Gesell. Pp 274: £20 hardback. Blackwell Scientific Publications, 1989. ISBN 0-632-02314-7.

The Yale Psycho-Clinic opened in 1911 for the assessment and treatment of children who had problems at school. It is principly associated with the name of Arnold Gesell who was responsible for a long series of observational studies in which the behaviour and movements of infants and children were painstakingly filmed, described, and recorded. He set norms for behaviour in the laboratory against which an individual could be assessed and in doing so laid the foundations of the developmental assessment techniques that are in use today the world over. It is therefore fitting that when the MacKeith Press launched a series of *Classics in Developmental Medicine* under the series editorship of Ross Mitchell they included among the first volumes a book by Gesell. Interestingly, they chose one of his later more theoretical and speculative books rather than one of the earliest 'atlases' of behaviour. *The Embryology of Behaviour*, subtitled The Beginnings of the Human Mind, was first published in 1945 and describes the development of behaviour in the fetus and infant. It draws on Gesell's own films of infants and the work of human developmental neurologists such as Wilhelm Preyer and Davenport Hooker as well as the galaxy of embryologists who were active in that period—such names as D'Archy Thompson, Barcroft, Kuo, Carmichael, Coghill, and Needham.

To the contemporary developmentalist, it is perhaps surprising to see how familiar the thinking is despite limitations of knowledge and techniques. For instance at that time direct studies of human fetal behaviour were limited to Davenport Hooker's films of dying embryos and it would be another 50 years before Heinz Prechtl would give us the chance to watch ultrasound films of babies in utero, yet Gesell understood the rudiments of fetal behaviour. Likewise, he discusses gene expression though it was his belief, like his contemporaries, that the genetic material was a protein. We also cannot fail to be impressed by the way this book moves so easily through the fields of biology, embryology, physiology, and psychology in a way that would indeed be rare today.

Perhaps for a neonatologist the most interesting thing about the book is that it gives an accurate picture of the knowledge and understanding of the fetus and newborn at exactly the point when neonatology was born. Indeed, it is reasonable to claim Gesell as one of the founders of neonatal paediatrics as his work was very important in drawing attention to the particular problems of the small and sick newborns.

M P M Richards
READER IN HUMAN DEVELOPMENT
University of Cambridge

The Sudden Infant Death Syndrome. Cardiac and respiratory mechanisms and interventions. Edited by P J Schwartz, D P Southall, M Valdes-Dapena. Pp 474: price not stated, paperback. Annals of the New York Academy of Sciences, 1988. ISBN 0–89766–463–9.

Many of the cognoscenti of the sudden infant death syndrome (SIDS) research fraternity gathered by the shores of Lake Como in May 1987 to discuss various aspects of sudden infant death. Their programme was organised by the three editors of this volume, which records the bulk of the deliberations. Papers were grouped into five main sections: an overview of the main problems, the identification and management of at risk infants, the provision of support for the families including an assessment of the value of home monitoring, and the possible role of cardiac and respiratory mechanisms respectively in causing death. Contributions were generally kept short to allow ample time for discussion, but alas the latter has not been included in the text. However, there are summaries of a series of workshops held towards the end of the meeting with the aim of reaching a consensus in the more contentious areas.

Also present in Como, and conducting their own meeting, were SIDS parents who now have an organisation known as SIDS Family International. As it is parents who have raised money for some of the research reported here, their presence was appropriate. They discussed, among other topics, the need for those dealing with the families at their time of crisis to receive special training, and deplored the fact that in some countries postmortem examination is either not routine in cases of sudden unexpected infant death, or is conducted to imperfect standards.

The scientific sessions do not seem to have engendered a great deal of new material, and many of the papers covered work already published. Results from the large United States National Institute of Child and Human Development Cooperative Epidemiological Study of Sudden Infant Death Syndrome were reported here, but many of the risk factors have been identified previously. What I found most instructive and interesting were the papers dealing with the gradual maturation of sleep state patterns and their interdependence with temperature and cardiorespiratory mechanisms. They made it easier to understand how a sudden failure of function can occur and leave no apparent pathological trace. But the fin workshops cautioned that much furth work was necessary to prove the respec ability and reproducibility of tests cardiorespiratory function, and to sta dardise laboratory measurements so th different studies can be more proper compared.

This volume will be a useful source references and perhaps the most up to da summary of a subject, which far from bei a single entity has so many facets that mu concern all paediatricians.

P A Davi
RETIRED PAEDIATRICI
Lond

Problems in Pediatric Emergency Medicin Edited by Robert C Luten. Pp 296: £26·
hardback. Churchill Livingstone, 1988.

This volume is the first in a series entitle *Contemporary Issues in Emergency Mec cine*. There are four chapters on gener issues, followed by nine on various types paediatric emergency, each by a differe author. Its scope and depth suggest that th American approach to the subject is mo professional than the British, several of tl authors being specialists in paediatr emergencies.

The early general chapters contain mar sensible observations and useful tip Luten, writing on recognition of the sic child, stresses the value of careful observ tion as compared with investigation pointing out that the more experienced tl paediatrician the fewer the tests. Simc goes further, saying of investigations th 'at their worst, they can mislead, dela cause needless pain and radiation exposur add to cost and even be responsible f clinical deterioration'. For smaller units l recommends the use of protocols f management and ready consultation wi and transfer to a larger centre. On tl practical side he commends the skill nurses in putting up drips and the use of tl intraosseus route, into the tibia, whe veins are inaccessible. The chapter on chil abuse, by Ludwig, is misplaced in th volume because it deals with causes an their remedy rather than recognition an management.

The remaining chapters cover traum poisoning, and acute illness in the maj systems. Their content is similar to th found in English textbooks, though oft more detailed and discursive. Transatlant differences emerge here and there: the u stalloid rather than colloid in shock; junction to start anticonvulsants after gle convulsion and an abnormal oencephalogram; recommendation of lised L-adrenalin for croup but no ion of ipratropium bromide for zy infants; strong advocacy of activated oal for various poisonings. But surely itive Babinski response is not flexor there (p 257)?

y paediatrician who has to deal with gencies will learn a lot from this book. ntended for digestion at leisure rather for quick reference in a crisis.

C J Bacon
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Friarage Hospital,
Northallerton

enital Diaphragmatic Hernia. Edited Puri. Pp 161: £54·60 hardback. Karger, . ISBN 3-8055-4807-9.

publication of this book is very timely ere is at present much emotive debate erning the optimum management for orns with diaphragmatic hernia. The r is to be congratulated on producing ost readable summary of the large ant literature in 13 chapters of uni- ly high standard. The initial chapters pidemiology, embryology, and patho- iology are essential before tackling the more clinical chapters.

ntres in four countries review their al management and the reader can are the results of immediate surgery, onged preoperative stabilisation, and corporeal membrane oxygenation. chapter on fetal surgery is confined to al work with only a tentative recom- dation for a trial in the human situation. intriguing to read that there is uncer- y from embryological models as to her lung hypoplasia is the primary rmality or secondary to the diaphrag- c defect.

e reader cannot fail to review his or wn views after reading this provocative authoritative text. The chapters are reference and there is an exhaustive x. This book is highly recommended personal bookshelves as well as for ries.

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Bronchopulmonary Dysplasia. Edited by Eduardo Bancalari and J Thomas Stocker. Pp 441: £50 hardback. Hemisphere Publishing Corporation, 1988. ISBN 0-89116-681-5.

This book provides an excellent review of a disease that has evolved greatly since Northway's first description. A balanced overview of the pathogenesis of bronchopulmonary dysplasia is followed by nine chapters, which in well referenced detail, examine the role of oxygen toxicity, barotrauma, inflammation and the use of animal models. Chapters on pathophysiology include histology, lung function, radiography, the upper airway, and pulmonary vascular components of this disease. It becomes clear that despite current respiratory mangement, which may include antioxidant treatment, surfactant, steroids, and alternative modes of ventilation, we are still inadequately avoiding morbidity and mortality. The final section has a discussion of long term prognosis, outpatient management, and ethical issues.

This book is easy to read, well indexed, and provides a good background for everyone dealing with the complexities of this respiratory disorder. A single concern is that statements on management are not always backed up: this is probably due to the inadequate number of good studies on treatment. For example it may not be appropriate to keep oxygen saturation between 85 and 95% as suggested, as the lower end of this range would include hypoxaemic levels This valued book highlights that this disease clearly needs further research into understanding, treating, and preventing what appears to be largely an iatrogenic problem.

M Samuels
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Paediatrics and Child Health. A Handbook for Health Professionals in the Third World. Edited by H M Coovadia and W E K Loening. 2nd ed. Pp 526: £8·95 softback. Oxford University Press, 1988. ISBN 0-19-570-445-2.

Within our shrinking world this comprehensive paediatric textbook from Africa is particularly useful. Not only does it cover virtually all common paediatric conditions seen in Europe, but also deals with the tropical infections that can enter Britain daily by that modern disease vector—the jumbo jet. The book comes from the University of Natal with a strong team of 30 authors. Despite its 526 pages it does not appear heavy in any sense and frequent line drawings and figures add interest and clarity. Photos are restricted to the dermatology section and most are useful despite being in black and white. It is clearly and sympathetically written and the first chapter on history taking and examining children, mainly for undergraduates, is excellent and aptly describes a paediatrician as one who 'must obtain genuine pleasure from examining and dealing with children'.

Inevitably there are some points of disagreement and apparent deficiencies. Many paediatricians, including the WHO advisors, no longer believe that diarrhoea with severe dehydration requires intravenous plasma expanders, blood, and bicarbonate, but Ringers' lactate in adequate amounts can correct both hypovolaemia and acidosis. A consideration of coma now appears incomplete without reference to a measure of unconsciousness like the Glasgow coma score. To cover all the routine immunisations in a page and a half means some important issues are glossed over. In many tropical countries seroconversion to oral poliovirus vaccine is poor. The schedule proposed (p 188) does not include the extra dose of this vaccine shortly after birth, which is the current WHO, Expanded Programme on Immunistion recommendation. Inadequate and inconsistent referencing is irritating. The bibliography is only to textbooks. In a number of places the authors refer to specific papers and studies, sometimes only by the author's name (for example, H Scott, p 134), sometimes without any reference (for example, p 121 a follow up study on kwashiorkor), and sometimes with reference but no authors (for example, tables 8.5 and 8.6 on p 163).

If you are bound for Africa this book could save you taking both a paediatric and a tropical medicine book. If children from Africa come to you in Britain, this is a ready reference that doesn't expect you to be a tropical expert.

W A M Cutting
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Edinburgh

Vascular Birthmarks—Hemangiomas and Malformations. By JB Mulliken and AE Young. Pp 483: £98 hardback. W B Saunders, 1988. ISBN 0–7216–6601–9.

This book is a blend of England New and Old, being the result of collaboration between two surgeons from Harvard and St Thomas's respectively. In the chapters they have written themselves they have achieved a uniformity of text and style if rather too American for my taste. Chapters from other contributors are less successfully incorporated.

The book is divided into four. Of part one I particularly enjoyed the (largely irrelevant) chapters on birthmarks in folklore, history, art, and literature. Not many textbooks travel from Cicero to Gorbachev via James II of Scotland or the 'Tragedies of Cymberline' (Shakespeare) to 'Blind Love' (Pritchett) via 'The Birthmark' (Hawthorn). Of real importance is the following chapter on classification which is *the* essential reading if full use is to be made of the book. The system proposed I found both logical and practical. For example, two axioms are quoted: 'Not all hemangiomas look like strawberries' with the corollary 'Not all strawberries are hemangiomas'.

Part two consists of three chapters on haemangiomas, part three has nine more on the much rarer vascular malformations. Separation into pathogenesis, clinical assessment, investigation, radiological assessment and the like, followed by separate chapters on capillary, venous and arterial, etc, make for a repetitive but comprehensive treatment of the subject.

Part four comprises eight more chapters dealing with the central nervous system and regions of the body—head and neck, upper limb, and so on. The final chapters deal with laser therapy, embolisation, and the emotional impact of a vascular birthmark on the family.

The book is well illustrated—in colour where appropriate. It deals with a difficult and wide ranging subject. The authors demonstrate an enthusiasm for and an encyclopaedic knowledge of the subject with an acute awareness of the need of patients and their parents.

The preface states this book is 'intended for a reference for both primary and secondary care physicians'. I think they include paediatricians among the former—I fear it fails, for it is necessary to read most of the book, to understand its layout, to know how and where to look up the problem. In places it is too detailed with an excess of background information and theory. I suspect it is the sort of book that many departments might think they should have but would rarely use. I consider it interesting reading rather than a usable reference work and it is too expensive for most individuals to buy. I would add that a plastic surgeon with a particular interest in this field might consider this book his bible on the subject.

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Textbook of Paediatric Anaesthetic Practice. Edited by Edward Sumner and David Hatch. Pp 603: £37·50 hardback. Harcourt Brace Jovanovich, 1989. ISBN 0–7020–1336–6.

To paraphrase Thurber, you can please some of the people all of the time, all of the people some of the time, but not all of the people all of the time, at least not very often! Hatch and Sumner's *Textbook of Paediatric Anaesthetic Practice* should please both the specialist paediatric anaesthetist and the general anaesthetist who deals with children.

The editors have commissioned chapters from 28 authors, mainly from North America, Australia, and Great Britain, to produce a comprehensive and authoritative textbook. Many of the chapters are written by experts in their field and include recent advances and a commendably up to date set of references. The text covers all aspects of paediatric anaesthesia from preoperative assessment to postoperative management and includes chapters on paediatric liver transplantation, paediatric heart-lung transplantation, and paediatric anaesthesia in developing countries. It seems unfair to single out chapters in a book with such a high overall standard, but the chapters on unusual conditions in paediatric anaesthesia by Anne Lynn and on resuscitation in paediatrics by David Zideman, will be particularly useful to many anaesthetists.

As many children in Britain are managed on adult intensive care units, at least until transfer can be arranged to a specialist centre, I think guidance on the principles of paediatric intensive care and on dealing with the more common conditions in paediatric intensive care would be a valuable addition to this textbook, particularly as few of the adult intensive care texts include chapters on paediatrics. The bo deals with anaesthetic practice, howeve and the editors may say intensive care w not in their remit.

In the preface the editors say the book aimed at the general anaesthetist who de with children, the anaesthetic trainee si ting for higher examinations, and th specialist paediatric anaesthetist. This is ambitious aim that is fulfilled. The text clear and readable with good illustratio and diagrams and very reasonably priced- there should be a copy in every departme where children are anaesthetised. It highly recommended.

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Maternal Fetal Medicine. Principles an Practice. Edited by R Creasy and R Resni Pp 1236: price not stated, hardbac W B Saunders, 1989. ISBN 0–7216–2304–

For those who like to have everythi under one cover this could well be th choice for a specialist book in advance postgraduate obstetrics. It is an extreme heavy book to carry and hopefully it wi not disappear easily from departments an libraries. Despite its size it is broken dow into over 50 chapters, some subdivided, s in no way is it an awesome book. It essentially an American book with only handful of the 50 contributors coming fro Britain, Canada, and Australia.

Basic sciences are covered with over 1 pages on reproductive genetics and th typical sections relating to physiologic and anatomical aspects of pregnancy. Ma of the more recent developments in fet medicine are covered in a 200 page sectio entitled 'Fetal diagnostic and treatme modalities'. The section has detailed u dated chapters on, for example, ultrasoun and Doppler. The fetal heart is considere not only in these two chapters but also in i own specific chapters relating to physiolog the fetal heart rate, and arrhythmias. Th latest proposals relating to fetal therapy a discussed in relation to this and oth organs.

The more conventional obstetric subjec —for example, prematurity—are in gener covered in succinct but readable, we referenced chapters. One or two parts a written somewhat parochially both in the approach and in their references but general I feel the book could easily fit in tish obstetric practice. The rest of the k covers maternal disorders of preg- cy—400 pages—almost a book unto lf. The editors have failed in this section curtail the ramblings of one or two hors both in their verbosity and their occupation with their own research. wever, in contrast there are some excel- t chapters in this section and the one on critically ill patient covers some useful as often missing in such texts.

or the obstetrician, this would be a d reference for those developing a cial interest in fetal medicine. The diatrician might find it useful for keeping obstetric colleagues on their toes.

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uromuscular Diseases. 2nd ed. By Swash and MS Schwartz. Pp 456: £99 dback. Springer-Verlag, London, 1988. N 0-387-19505-x.

is is the second edition of this successful k on diagnosis and management. The book has been considerably expanded and has now developed into a quite formidable tome. There are the expected chapters on the inherited disorders including the muscular dystrophies, the spinal muscular atrophies, the myotonic disorders, and the congenital myopathies, to name just a few. In addition there are chapters on mononeuropathies, nerve root and plexus lesions, acquired polyneuropathies, the myasthenic syndrome, inflammatory disorders, endocrine myopathies, toxic myopathies, cardiomyopathies, and other clinical problems.

The text is clear, clinical descriptions are excellent, as is advice on differential diagnosis. Clinical photographs are well chosen. One of the authors has an interest in muscle and nerve pathology and the book contains many excellent photomicrographs and electronmicrographs. The other author has an interest in neurophysiology, and as well as a separate chapter on this subject, the text is peppered with useful advice on electrodiagnosis.

The book is impressively comprehensive, such that the chapter on disorders of the anterior horn cell deals not only with the common childhood types of spinal muscular atrophy but also mentions nine rare forms. The muscular dystrophies get similar treatment. The chapters on the mononeuropathies and the brachial and lumbar nerve root lesions are welcome. These are rare in childhood, are difficult to investigate, and it is useful to find comprehensive coverage of them.

Despite this extensive coverage, the book is not specifically paediatrically orientated. Thus sections of physical management and rehabilitation are not comprehensive. I cannot agree with the recommendation on steroid management of juvenile dermatomyositis, and the literature is not adequately quoted on this most important subject. The otherwise excellent chapter on myasthenia devotes relatively little space to the congenital syndromes. The authors recommend open muscle biopsy, which is going out of fashion in children, although they discuss needle biopsy. The emphasis on electromyography is not so practical when investigating children and the authors dismiss ultrasound imaging of muscle as an alternative. They do not, for example, discuss the problems of investigating patients with selective muscle involvement.

This book is noteworthy for its excellent and comprehensive coverage of neuromuscular disorders, which are less common in childhood but are a major problem of management when they do occur. It complements rather than replaces existing paediatric texts on the subject.

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Archives of Disease in Childhood, 1989, **64**, 1526

Neonatology—then and now *(C H M Walker)*

Vitamin K (1961)

On real and apparent external bleeding in the newborn

W S CRAIG

*Department of Paediatrics and Child Health, University of Leeds (Arch Dis Child 1961;**36**:575–86)*

The late WS Craig records the anatomical site of bleeding in 345 infants seen over a period of 11 years—a considerable clerical task made easier today by the use of computers and provided the initial observations and recordings are accurate! The most common site was gastrointestinal (155), followed by cutaneous and subcutaneous (96), cord/umbilicus (26), and haematuria (23). There were fewer than 20 cases each of superficial trauma, haemoptosis, bucconasopharyngeal bleeding, and after circumcision. Seventy four babies had bleeding from more than one site, five of which bled from three sites.

The principal interest in this paper today is the reference made to the routine use of vitamin K. Despite enthusiastic claims by others of the 'virtual disappearance of haemorrhagic disease' with this treatment Professor Craig remained unconvinced. He found evidence of coagulation defect in only 24 of his 345 cases and among the 52 deaths haemorrhage was the primary cause in only five.

He agreed with Wintrobe (1951) in thinking that the condition was self limited and argued that:

> 'If coagulation defect is the only aetiological operating factor it is difficult to understand why simultaneous bleedings in multiple sites should not be of commoner occurrence (Quick, 1942)'.

He finishes with the comment:

> 'Reasons are advanced for questioning the value of vitamin K therapy in the prophylactic and curative treatment of primary intrinsic gastrointestinal haemorrhage of the newborn'.

Today The debate about giving every newborn baby vitamin K continues. This may in part be due to the varying incidence and severity of vitamin K dependent haemorrhage from place to place in the United Kingdom. My personal experience led me to resist this suggestion as over a period of at least 10 years I saw only one or two cases of significant vitamin K dependent haemorrhage per year with no deaths.

This has not been the experience in other parts of the United Kingdom, however, so it is now usually recommended that all babies are given routine treatment, especially as vitamin K_1 can now be given orally (though with slightly less assurance of bioavailability). An acceptable compromise has been suggested by Tripp and McNinch.[1]

> 'For reasons of acceptability to parents, safety, convenience, and cost we currently use 1 mg oral dose of vitamin K_1 for routine prophylaxis and continue to use intramuscular prophylaxis in infants at special risk from HDN [haemorrhagic disease of the newborn]. . . . We have now used this policy in some 25 000 babies and have not seen any further cases of HDN'.

While this is very gratifying, I am not alone in wondering why this has become necessary. Surely our efforts should be directed towards finding the cause of the deficiency and assessing the potential of preventive antenatal treatment.

Reference

[1] Tripp JH, McNinch AW. Haemorrhagic disease and vitamin K. *Arch Dis Child* 1987;**62**:436–7.

The late WS Craig, Fellow of the London and Edinburgh Colleges of Physicians and of the Royal Society of Edinburgh, first graduated as a naval architect in 1924. Dissatisfied with his work in a Glasgow shipyard he turned to medicine, graduating in Edinburgh in 1930. His calibre was already manifest in that he had been Buchanan Scholar and James Thomson medallist and soon became assistant to Professor Charles McNeil in the newly formed Edinburgh University Department of Child Life and Health. Income from such a post in those days was miniscule and he found it necessary to move for financial reasons, taking up a post in the Ministry of Health. He worked there for 10 years but always maintained a close contact and interest in hospital paediatrics, eventually becoming the first Professor of Paediatrics and Child Health, University of Leeds in 1946. In the same year his book *Child and Adolescent Life in Health and Disease* was published. In 1952 he started one of the earliest special care baby units in Leeds Maternity Hospital and he is probably best known today for his book *Care of the Newly Born Infant* (1956) the first four editions of which he produced and which, in updated form, is still a standard on the subject today. He has been described to me by one who knew him well as a fine clinician and administrator who sometimes showed 'a fair degree of cussedness' and who had difficulty in being patient with those who showed a lack of commonsense. He was, however, 'a man of powerful virtues—always a memorable man'.

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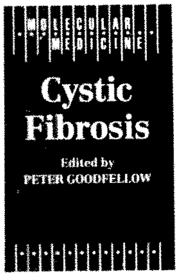

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Archives of Disease in Childhood, 1989, **64**, 1647–1648

Annotations

The cystic fibrosis gene

In a clutch of three papers from a recent issue of *Science* scientists from Toronto and Michigan describe the isolation, sequence, and major mutation of the gene at the cystic fibrosis locus.[1-3] These reports represent the culmination of several years painstaking work by many scientists worldwide and herald a new era of multidisciplinary effort aimed at devising a treatment for this life limiting condition.

The gene itself was pinned down by extensive genetic analysis of families with cystic fibrosis from many countries; it is likely that the limits of genetic resolution had almost been reached. With the gene bracketed by two DNA markers Dr Lap-Chee Tsui (Hospital for Sick Children, Toronto), Dr Francis Collins (University of Michigan), and their collaborators utilised the technique of 'chromosome jumping'. This allowed them to move over large distances of a chromosome (of the order of 100 000 basepairs of DNA, 100 kb) relatively quickly. After every jump the position of each clone with respect to the gene defective in cystic fibrosis (*CF*) was checked using genetic and physical mapping methods. Clones close to *CF* were tested for the presence of genes which were expressed in tissues known to be affected by the disease; one gene was isolated which mapped to the area suspected of harbouring *CF* and which was expressed in lung, liver, sweat gland, colon, and pancreas.

The candidate gene is large, extending over 250 kb of genomic DNA encoding an mRNA of 6·5 kb constructed from 24 exons (regions of translated sequence separated by non-coding introns). In order to obtain further evidence that this might be *CF* the sequence of the gene from affected and heterozygote individuals was compared and a deletion of three base pairs of DNA was found in the sequence from chromosomes carrying *CF*. The deletion was found to alter the structure of a protein involved in membrane transport processes.

The sequencing data yielded much other interesting data. The predicted protein sequence of the gene (dubbed cystic fibrosis transmembrane conductance regulator, CFTR) is similar to that of a known gene family encoding the P-glycoproteins alternatively known as multiple drug resistance (MDR) proteins. These proteins were isolated in cells that had become resistant to high concentrations of cytotoxic drugs where they are expressed in huge amounts secondary to gene amplification. The MDR genes encode proteins that span the cell membrane and pump the toxic agents out of the cell in an ATP dependent fashion. The function of the MDR family in the normal cell is unknown; one possibility is that they excrete toxic organic compounds from the cell, another is that they export proteins from the cell.

The discovery of a transmembrane protein encoded at *CF* is not a complete surprise; a series of elegant experiments has demonstrated a deficiency in chloride ion permeability in the apical membranes of epithelia in cystic fibrosis. After certain (non-physiological) stimuli the channel for chloride ions found in membranes from cystic fibrosis patients can be made to function in an essentially normal way, demonstrating that the anion transporting domain of the protein is intact but that the regulation of its activity is defective.[4] CFTR may be a novel type of channel which transports chloride, and perhaps other substrates, in response to intracellular stimuli such as raised cAMP levels and protein kinase activity. Alternatively, the protein may not itself transport chloride, but regulate the activity of the protein that does. The three base pairs at the deletion found in cystic fibrosis encodes a phenylalanine residue which will be absent from the protein in cells in cystic fibrosis; the mutation leaves the rest of the mRNA in frame for translation so that the remainder of the protein is intact. One current hypothesis is that the deletion of this single amino acid results in a conformational change that renders the protein refractory to the various activators of chloride permeability.

It was already thought likely from previous studies that two or more different mutations, in the same gene, may cause cystic fibrosis.[5 6] The sequencing of CFTR from various people with cystic fibrosis supports this view. A total of 68% of chromosomes carrying *CF* had the 3bp deletion in CFTR; work is currently underway to determine the nature of the other mutations. The 3bp deletion was never found on the normal chromosomes of obligate heterozygotes. It is of interest that patients with cystic fibrosis who have pancreatic sufficiency tend to have one chromosome with the 3bp deletion and one chromosome with another, as yet unknown, mutation: that is, they are compound heterozygotes. Patients with pancreatic insufficiency are very often homozygous for the 3bp deletion, suggesting that either this mutation alters protein function more severely than the others or that the Phe residue is particularly important for functional regulation in the pancreas.

At the clinical level the immediate benefits of this advance are, once all the mutations are characterised, antenatal diagnosis with a theoretical accuracy of 100% and carrier detection for random members of the population wishing to marry those known to be heterozygous for cystic fibrosis. In the same way it will be possible to confirm a diagnosis of cystic fibrosis in doubtful cases. Diagnostic difficulty, however, is more likely to occur in milder forms of the disease for which the mutation has not yet been determined. With the availability of the polymerase chain reaction it is now possible to detect the major mutation in cystic fibrosis using minute quantities of blood. Should this procedure become automated a method of neonatal screening/heterozygote detection would be feasible using the blood on a Guthrie card. With the necessary political and financial will it will be possible to introduce population screening for the cystic fibrosis mutation, which may make a large impact in reducing the incidence of the disease in the future.

In the longer term the aims will be to devise a treatment for existing and future patients with cystic fibrosis. Pharmacological agents will be sought that modify the activity of CFTR and restore apical membrane chloride permeability, although this is likely to be a difficult task. Several groups will investigate the possibility of introducing a normal CFTR gene into affected cells using viruses tropic for the cell type in question. Mouse models of cystic fibrosis will need to be created so that any new therapeutic approach can be tested in animals. This will be attempted by mutating the mouse homologue of CFTR and breeding mice homozygous for the cystic fibrosis mutation.

As is usual in these situations this latest break through raises more questions than it answers, and certainly there is no prospect of an early 'cure' for cystic fibrosis. There are almost immediate clinical benefits in the areas of diagnosis and heterozygote detection, however, and at least we now know what questions to ask in the search for a complete understanding of the pathophysiology and eventual effective treatment of cystic fibrosis.

References

[1] Rommens JM, Iannuzzi MC, Kerem B-S, *et al.* Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science* 1989;**245:**1059–65.

[2] Riordan JR, Rommens JM, Kerem B-S, *et al.* Identification of the cystic fibrosis gene: cloning and characterisation of complementary DNA. *Science* 1989;**245:**1066–73.

[3] Kerem B-S, Rommens JM, Buchanan JA, *et al.* Identification of the cystic fibrosis gene: genetic analysis. *Science* 1989;**245:** 1073–9.

[4] Schoumacher RA, Shoemaker RL, Halm DR, Tallant EA, Wallace RW, Frizzell RA. Phosphorylation fails to activate chloride channels from cystic fibrosis airway cells. *Nature* 1987;**330:**752–4.

[5] Estivill X, Scambler PJ, Wainwright BJ, *et al.* Patterns of polymorphism and linkage disequilibrium for cystic fibrosis. *Genomics* 1987;**1:**257–63.

[6] Kerem B-S, Buchanan JA, Durie P, *et al.* DNA marker haplotype association with pancreatic sufficiency in cystic fibrosis. *Am J Hum Genet* 1989;**44:**827–34.

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Immunity to viruses

The traditional view of immunity to viruses has heavily emphasised the role of serum and secretory antibody and neglected the role of cellular immunity and non-immune host defences such as interferons.[1] Boys with isolated antibody deficiency (X linked hypogammaglobulinaemia) and normal or near normal cellular immunity, however, recover normally from most virus infections with the exception of enteroviruses (echo and coxsackieviruses can cause persistent, often fatal, central nervous system and muscle infection and there is an increased risk of paralytic poliomyelitis). In contrast, children with isolated deficiencies of cellular immunity[2] and those with acquired deficiencies of cellular immunity but normal antibody production, such as children on cytotoxic or immunosuppressive treatment, develop severe and often fatal infections with measles, chickenpox, respiratory syncytial, influenza, and parainfluenza viruses.[3]

Role of antibody

Antibody is important in *preventing* viral infection and reinfection but far less important in *recovery* from acute infection. Antibodies are produced by B lymphocytes or B cells, which have been activated to plasma cells. Such B cell activation requires assistance from 'helper' cells called helper T lymphocytes or T helper (T_H) cells. Acute infection results in serum IgM antibody production followed by serum IgG. Secretory IgA is produced locally in response to infection, although IgA production is poor in the neonatal period and early infancy. These antibodies are probably of only minor importance in recovery from viral infection, as antibody deficient individuals can recover normally. For example, nasal secretory antibody against respiratory viruses generally appears too late to limit viral replication.[3–6]

Antibody does, however, seem to be important in preventing infection or at least decreasing the severity. Transplacental IgG protects neonates and infants against virus infection. Passive administration of hyperimmune gammaglobulin against varicella zoster virus or measles virus decreases the severity if not the incidence of infection in newborns or immunocompromised subjects.[7] The presence of pre-existing specific nasal IgA correlates better than serum antibody with protection against respiratory viral infection.[5 7] IgA deficiency is usually found coincidentally in normals, but may also be associated with recurrent infections of the respiratory and gastrointestinal tracts. Deficiencies of IgG subclasses, particularly IgG_2, are also associated with recurrent respiratory tract infections.

Cellular immunity

(1) T CELLS AND ANTIGEN PROCESSING

Thymus derived or T lymphocytes (T cells) are responsible for cellular or cell mediated immunity. They will only recognise foreign antigens, however, which have been 'processed' and presented on the surface of antigen presenting cells. Almost any cell can act as an antigen presenting cell, but macrophages and monocytes are the most extensively studied. Macrophages recognise foreign antigens, ingest them and 'process' them. T cells only recognise antigens presented by cells which share the same HLA type. It is now known that macrophages present processed antigen on the surface of the cell, in intimate conjunction with one of their major histocompatibility complex, also called HLA antigens. Thus the T cell response is said to be major histocompatibility complex restricted.

In elegant studies on the recognition by T cells of influenza virus infected cells, Townsend and Skehel have shown that it is not the surface proteins of influenza virus that are primarily recognised but the nucleoprotein.[8] This seems intuitively wrong as nucleoprotein would be expected to be expressed in the nucleus of infected cells, not on the cell surface. Townsend went on to show, however, that the T cell is actually recognising short peptides of denatured influenza virus nucleoprotein, only about 14 amino acids long, expressed on the cell surface.[9] Similar recognition of viral peptides by cytotoxic T lymphocytes has since been shown for human immunodeficiency virus.[10] Thus it seems that antigen presenting cells can break down viral proteins and express one or more of the resulting peptides on their surface for T cells to recognise. It now appears that the peptide lies in a groove in the major histocompatibility complex molecule on the antigen presenting cell's surface where it can be recognised by T cells[11]; if a single amino acid in the peptide is changed this may alter its shape so it will not fit in the groove.

The T cell recognises the viral peptide through a specific T cell receptor, which has constant and variable regions that can be thought of as analogous to immunoglobulin (fig 1). It becomes irreversibly 'committed', discards the DNA for other receptors, and starts to proliferate. It then can mature either into a memory cell, which becomes quiescent but recognises the same antigen if re-exposed, or into an effector cell. These effector cells can either destroy infected cells (cytotoxic T cells) or help B cells to produce antibody (T helper cells). T cells also release soluble proteins, lymphokines, such as interferon gamma (see below), which recruit inflammatory cells and thus limit the spread of infection.

Cytotoxic T cells seem to be important in protection against influenza virus infection[12] and in recovery from bronchiolitis caused by respiratory syncytial virus.[13] However, they may also contribute to disease. Cytotoxic T lymphocytes are found in the blood and cerebrospinal fluid in mumps meningitis, and a pathogenic role has been suggested.[4]

(2) NATURAL KILLER CELLS
Natural killer cells resemble large granular lymphocytes morphologically, but probably come from a lineage distinct from B and T cells. They can kill virus infected cells, as well as tumour cells and some micro-organisms. This is not an immune mechanism as it occurs spontaneously without prior exposure, and does not require antigen processing or major histocompatibility complex recognition. Natural killer cells are stimulated by both interferon alfa and interferon gamma (see below) and become more active. The importance of natural killer cell activity in different viral infections varies: natural killer cells seem to be particularly important in limiting infections due to herpes viruses.[15]

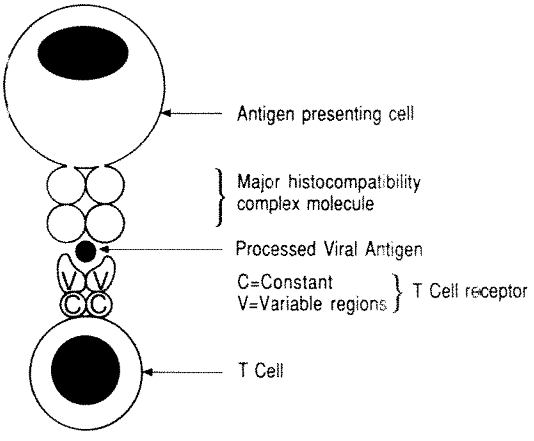

Fig 1 *Diagrammatic representation of the recognition by a cytotoxic T cell of viral peptide presented by an antigen presenting cell in association with a major histocompatibility complex molecule. (The illustration is from Isaacs D, Moxon R, Neonatal Infections; this is to be published by Butterworths in late 1990.)*

(3) ANTIBODY DEPENDENT CELLULAR CYTOTOXICITY
The role of antibody dependent cellular cytotoxicity in viral infections is unclear. The responsible cells, predominantly lymphocytes, recognise viral antigen bound to antibody and will then kill virus infected cells. Like natural killer cells they are activated by interferon gamma.

Interferons

There are three types of interferon: alfa, beta, and gamma.[16] Interferon alfa can be produced by almost any nucleated cell in the body and interferon beta by fibroblasts and epithelial cells in response to various stimuli, particularly virus infection. They then act on neighbouring cells, stimulating them to produce various antiviral proteins that render them resistant to viral infection. This is not an immune mechanism: interferons alfa and beta can be produced in response to a wide range of viruses and also protect against a wide range of viruses.

Interferon alfa is produced locally in most respiratory virus infections before antibody can be detected and is probably one of the most important mechanisms of recovery from such infections. In general interferon alfa is not produced by the body in the absence of infection, although bronchoalveolar lavage of healthy volunteers has shown high resting lung concentrations of interferon alfa, a finding of uncertain significance.[17] If mice are pretreated with anti-interferon alfa antiserum they become far more susceptible to infection with herpes simplex virus, encephalomyocarditis virus and other viruses, though not to influenza virus.[18 19] Interferon alfa does not appear to be important in recovery from respiratory syncytial virus infection.[20] Children with defective production of interferon alfa, apparently due to delayed maturation of production, however, have an increased incidence of respiratory viral infections with wheeze.[21]

Interferon gamma, or immune interferon, in contrast to interferon alfa, is produced by T cells on re-exposure to an antigen to which they have already been sensitised and is thus an immune mechanism. Although interferon gamma has antiviral properties, it appears to have a more important role as a modulator of the immune response. Macrophages and monocytes are activated by interferon

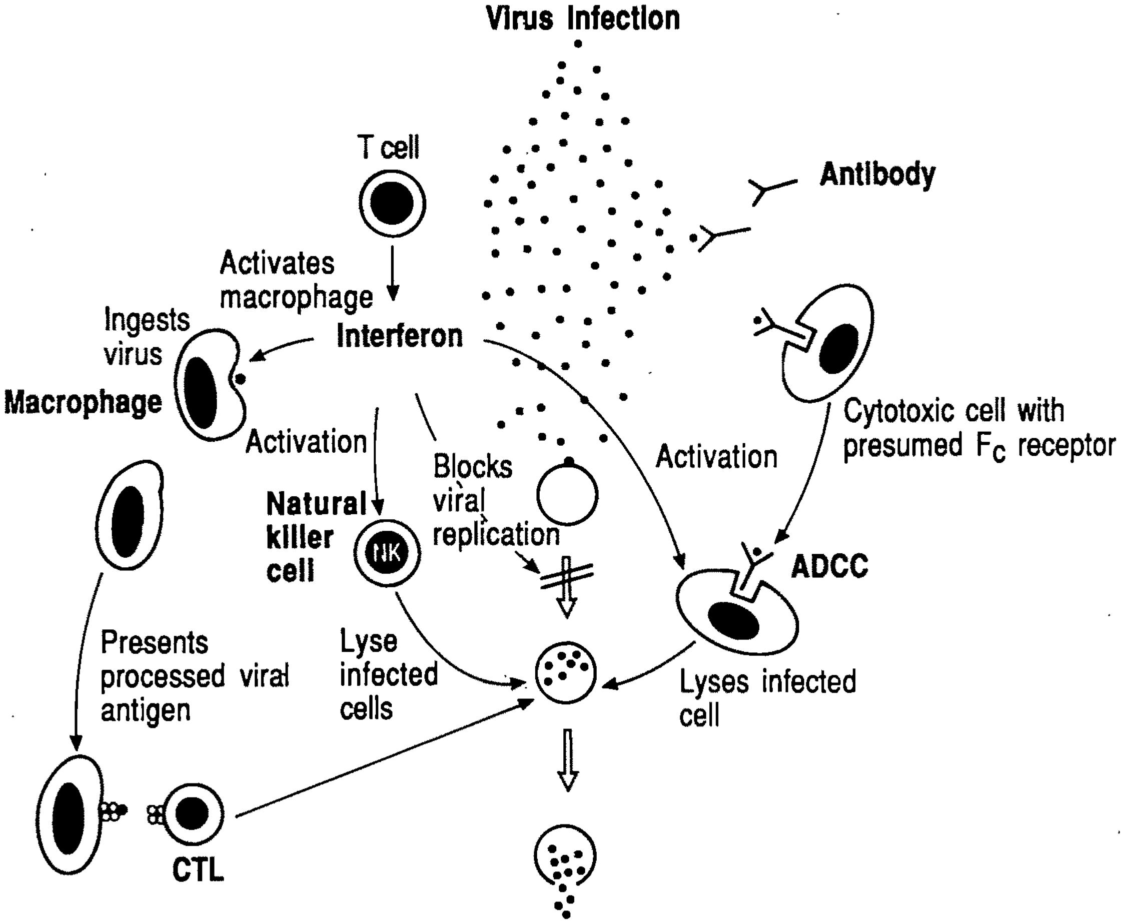

Fig 2 *Interaction of various host defence mechanisms in recovery from viral infection. ADCC=antibody dependent cellular cytotoxicity, CTL=cytotoxic T lymphocytes. (The illustration is from Isaacs D, Moxón R, Neonatal Infections; this is to be published by Butterworths in late 1990.)*

gamma, thus improving their ability to process and present antigen, while natural killer cells and the cells responsible for antibody dependent cellular cytotoxicity are activated to increase lysis of virus infected cells.[16]

Neutrophils

There is evidence that neutrophils are important in some virus infections, although neutropenia is more often found as a consequence of virus infections than it is recognised as a cause of them. Nevertheless, a neutrophil leucocytosis is seen early in experimental infections with rhinovirus, influenza, and coxsackieviruses and late in adenovirus infections. Some viruses—for example, herpes simplex and mumps—generate chemotaxins, and neutrophils can adhere to virus infected cells in vitro. Many different viruses have been identified within neutrophils and can activate neutrophil oxidative metabolism and intracellular killing.[22]

Conclusion

The defence mechanisms that have been described are of varying importance in different virus infections. It is becoming increasingly clear that the different mechanisms can interact with each other to amplify the immune response to virus infection. The release of lymphokines from activated T cells will activate a number of different cells to improve their ability to limit the spread of infection (fig 2).

I should like to express my gratitude to the Wellcome Trust for their support, and to Andrew McMichael, Charles Bangham, Alain Townsend, Frances Gotch, and others in the Nuffield Department of Medicine who have stimulated my interest and tolerated my ignorance; I also thank Miss Gail Davies for typing the text.

References

[1] Ogra PL, Morag A, Ogra SS, Beutner KR. Host defense mechanisms in viral respiratory infections. *Pediatr Res* 1977;**11**:231–3.

[2] Fishaut M, Tubergen D, McIntosh K. Cellular response to respiratory viruses with particular reference to children with disorders of cell-mediated immunity. *Pediatrics* 1980;**96**:179–86.

[3] Alford RH, Rossen RD, Butler WT, Kasel JA. Neutralizing and haemagglutination-inhibiting activity of nasal secretions following experimental human infection with A2 influenza virus. *J Immunol* 1967;**98**:724–31.

[4] Butler WT, Waldman TA, Rossen RD, Douglas RG, Couch RB. Changes in IgA and IgG concentrations in nasal secretions prior to the appearance of antibody during viral respiratory infections in man. *J Immunol* 1970;**105**:584–91.

[5] Cate TR, Rossen RD, Douglas RG, Butler WT, Couch RB. The role of nasal secretion and serum antibody in the rhinovirus common cold. *Am J Epidemiol* 1966;**84**:352–63.

[6] Rossen RD, Butler WT, Cate TR, Szwed CF, Cough RB. Protein composition of nasal secretion during respiratory virus infection. *Proc Soc Exp Biol Med* 1965;**119**:1169–76.

[7] Rossen RD, Butler WT, Waldman RH, *et al*. The proteins in nasal secretion. II. A longitudinal study of IgA and neutralising antibody levels in nasal washing from men infected with influenza virus. *JAMA* 1970;**211**:1157–61.

[8] Townsend ARM, Skehel JJ. The influenza A virus nucleoprotein gene controls the induction of both subtype specific and cross-reactive cytotoxic T-cells. *J Exp Med* 1984;**160**:552–63.

[9] Townsend ARM, Rothbard J, Gotch FM, Bahadur G, Wraith D, McMichael AJ. The epitopes of influenza nucleoprotein recognized by cytotoxic T lymphocytes can be defined with short synthetic peptides. *Cell* 1986;**44**:959–68.

[10] Nixon DF, Townsend ARM, Elvin JG, Rizza CR, Galloway J, McMichael AJ. HIV-1 GAG specific cytotoxic lymphocytes defined with recombinant vaccinia virus and synthetic peptides. *Nature* 1988;**336**:484–7.

[11] Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC. Structure of the human class I histocompatibility antigen, HLA-A2. *Nature* 1987;**329**:506–12.

[12] McMichael AJ, Gotch FM, Noble GR, Beare PAS. Cytotoxic T-cell immunity to influenza. *N Engl J Med* 1983;**309**:13–7.

[13] Isaacs D, Bangham CRM, McMichael AJ. Cell-mediated cytotoxic response to respiratory syncytial virus in infants with bronchiolitis. *Lancet* 1987;**ii**:769–71.

[14] Kreth HW, Kress L, Ott HF, Eckert G. Demonstration of primary cytotoxic T-cells in venous blood and cerebrospinal fluid of children with mumps meningitis. *J Immunol* 1982;**128**:2411–5.

[15] Biron CA, Byron KS, Sullivan JL. Severe herpesvirus infections in an adolescent without natural killer cells. *N Engl J Med* 1989;**320**:1731–5.

[16] Balkwill FR. Interferons. *Lancet* 1989;**i**:1060–3.

[17] Prior C, Haslam PL. Interferons in lungs. *Lancet* 1989;**i**:1333.

[18] Gresser I, Tovey MG, Bandu MT, Maury C, Bronty-Boye D. Role of interferon in the pathogenesis of virus diseases in mice as demonstrated by the use of anti-interferon serum. I. Rapid evolution of encephalomyocarditis virus infection. *J Exp Med* 1976;**144**:1305–15.

[19] Gresser I, Tovey MG, Maury C, Bandu MT. Role of interferon in the pathogenesis of virus diseases in mice as demonstrated by the use of anti-interferon serum. II. Studies with herpes simplex, Moloney sarcoma, vesicular stomatitis, Newcastle disease and influenza viruses. *J Exp Med* 1976;**144**:1316–27.

[20] Isaacs D. Production of interferon in respiratory syncytial virus bronchiolitis. *Arch Dis Child* 1989;**64**:92–5.

[21] Isaacs D, Clarke JR, Tyrrell DAJ, Webster ADB, Valman HB. Deficient production of leucocyte interferon (interferon-alpha) in vitro and in vivo in children with recurrent respiratory tract infections. *Lancet* 1981;**ii**:950–2.

[22] Faden H, Ogra P. Neutrophils and antiviral defense. *Pediatr Infect Dis* 1986;**5**:86–92.

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See related papers on p 1656 and p 1661.

Archives of Disease in Childhood, 1989, **64,** 1653–1655

Arteriovenous malformations involving the vein of Galen

Vein of Galen aneurysms are rare but since the first description in 1937[1] nearly 250 cases have been reported. They have been described as both aneurysms and arteriovenous malformations involving the Galenic system. The number of variants is in favour of the latter description.

Anatomy

The vein of Galen is the largest of the deep cerebral veins formed posterior to the pineal body by confluence of the two internal cerebral veins. It curves sharply upward around the splenium of the corpus collosum and drains the deep medially located veins of the diencephalon, basal ganglia, and mid brain.

Dilatation of the vein of Galen results from the force of arterial blood either directly from an artery or by way of a tributary vein or veins which receive blood from an artery.[2] The dilated vein of Galen is therefore a varix associated with an arteriovenous malformation. In most cases a venous (dural) anomaly can be demonstrated downstream from the draining vein,[3] and this, together with the high blood flow into the vein of Galen causes its dilatation. Hoffman *et al*[4] have described four prominent angiographic patterns of Galenic arteriovenous malformations and related these to the clinical presentation treatment and outcome.

PATTERN I

Many vessels including anterior cerebral arteries, thalamic perforating arteries, and superior cerebellar arteries discharge into the vein of Galen.

PATTERN II

A single posterior choroidal artery draining into the vein of Galen.

PATTERN III

One or both posterior choroidal plus one or both anterior cerebral arteries drain directly into the Galenic system.

PATTERN IV

An angiomatous network of posterior choroidal and thalamic perforating arteries enter the vein of Galen directly.

We have recently seen a fifth pattern in which a high flow arteriovenous malformation in the right inferior frontal lobe drained via the inferior sagittal sinus and pericallosal vein into the vein of Galen (fig 1). Embryologically these patterns are explained by the persistance of fistulae between primitive pia arachnoidal arteries and pial veins which cross each other at right angles. The primitive Galenic system lies close to the primitive choroidal system so an arteriovenous malformation involving these arteries will inevitably involve the Galenic vein.[5]

Clinical presentation

Four clinical syndromes have been described.[6] Each of these is associated with a different pattern of

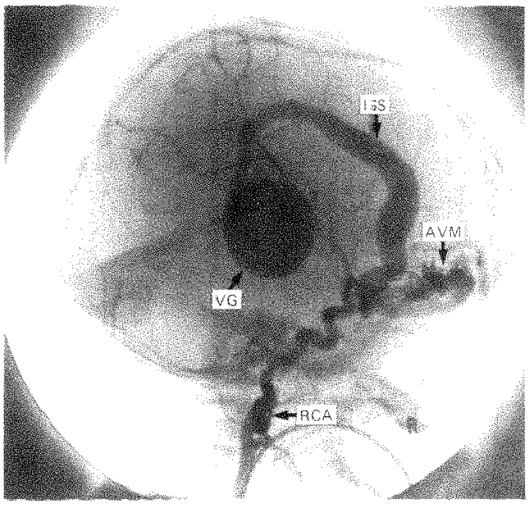

Fig 1 *Right carotid arteriogram. Right frontal arteriovenous malformation (AVM) draining via the inferior sagittal sinus (ISS) into a dilated vein of Galen (VG). RCA = right internal carotid artery*

Galenic arteriovenous malformation.[4] The size of the fistula dictates whether haemodynamic features predominate over local mass effect. The larger the arteriovenous shunt, the greater the haemodynamic effect and the earlier the patient presents.

Neonates presenting with severe cardiac failure and often a cranial bruit (pattern I) account for 44% of cases. These children are rarely hydrocephalic and subarachnoid haemorrhage is uncommon. The heart failure that occurs in this group is due to the size of the arteriovenous shunt which can steal 80% or more of the cardiac output, with large volumes of blood under high pressure returning to the right heart and pulmonary circulation.

Infants presenting with hydrocephalus, often a cranial bruit, occasionally epilepsy, and sometimes a progressive neurological defect caused by 'steal' phenomenon and compression of brain parenchyma by the aneurysm (patterns II and III) account for 30% of cases. The hydrocephalus is caused mainly by the mechanical effect of the dilated thick walled vein of Galen on the aqueduct of Sylvius. Communicating hydrocephalus can also occur if the venous (dural) obstruction is remote from the vein of Galen, thus affecting cerebral venous drainage and return of cerebrospinal fluid.

Children and adults presenting with headache, exercise syncope, neurological deterioration, cranial venous hypertension (distended scalp and facial veins plus epistaxis), and subarachnoid haemorrhage (pattern IV) account for 26% of cases.

More unusual presentations include various psychoses[7 8] and precocious puberty. Subarachnoid haemorrhage is relatively rare, occurring in 12% of reported cases. This is because of the great thickness of the wall of the dilated vein of Galen. When haemorrhage does occur it is often in young adults with a pattern IV arteriovenous malformation, the bleeding coming from the angiomatous arteriovenous malformation.

Diagnosis

The recognition of the presenting features, in particular cranial bruits and distended scalp and face veins, is the basis of diagnosis. However, these are not always present.[9]

In children with a patient anterior fontanelle, ultrasound is a sensitive imaging procedure,[10] and is the investigation of first choice (fig 2). Ultrasound has enabled prenatal diagnosis.[11] The use of Doppler ultrasound allows specificity by detecting blood flow,[10] and if it shows the typical features, cranial computed tomography can be avoided. If, however, the anterior fontanelle is closed or Doppler ultrasound unavailable, then contrast enhanced cranial computed tomography should be performed. Carotid and vertebral angiography is always necessary to determine the anatomy as this will affect the surgical approach and treatment. Magnetic resonance imaging may replace angiography because of its excellent imaging ability, high resolution, and capacity to image blood flow non-invasively.[12]

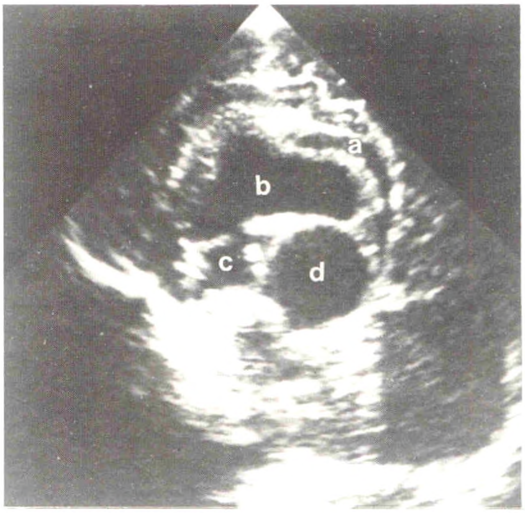

Fig 2 *Sagittal ultrasound scan, midline. a=Inferior sagittal sinus; b=dilated lateral ventricle; c=third ventricle; and d=dilated vein of Galen.*

Treatment and outcome

Supportive treatment is necessary initially. Thereafter treatment is surgical with an increasingly important contribution from interventional radiology. The overall mortality is 56%. Surgical mortality is 37% with 56% serious morbidity. There have been no reports of normal survival in untreated patients.

Management depends on the angiographic pattern. In most cases it is necessary to clip the fistulous tract from the arterial side into the vein. Postoperatively the varix progressively shrinks. In children with hydrocephalus a drainage procedure is also necessary until the vein has shrunk. However, when the arteriovenous malformation is distant to the Galenic vein excision of the malformation is also necessary. It has also been reported that shunting alone can result in reduction in size of the varix.[13]

In neonates with pattern I Galenic arteriovenous malformations there is a 91% mortality after surgery.[9] This is because the children are critically ill and in congestive cardiac failure. Myocardial infarction is common due to appreciably reduced coronary blood flow, and previously survivors were those in whom hypotension was avoided during surgery.[4] However, it is now possible to embolise the arteriovenous malformation percutaneously under radiological control using giant urco coils,[14] reducing flow sufficiently to correct heart failure, and allowing elective surgery when the child is older and fitter. Where this is possible there is a significant reduction in mortality and morbidity.[9]

In older infants and children with pattern II, III, and IV Galenic arteriovenous malformations the mortality after surgery varies between 26% and 32%. Postsurgical serious morbidity in these groups varies between 42% and 50% depending on preoperative effects of the malformation and postsurgical complications. In infants with a pattern II arteriovenous malformation and a single feeding artery, embolisation may be effective, reducing both morbidity and mortality.[9]

Conclusion

Arteriovenous malformations involving the vein of Galen are being seen with increasing frequency due to improved diagnostic techniques and awareness of the entity. In neonates early treatment is vital to prevent the blood supply to the cerebral parenchyma and myocardium being compromised by the flow through the malformation. In some cases this can be done effectively by embolisation. Early diagnosis and treatment in older children will similarly reduce serious morbidity and mortality. A multidisciplinary approach to investigation and management allows for satisfactory treatment in many patients.

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References

[1] Jaeger JR, Forbes RP, Dandy WE. Bilateral congenital cerebral arteriovenous communication aneurysm. *Transactions of the American Neurology Association* 1937;**63**:173–6.

[2] 'O'Brien MS, Schecter MM. Arteriovenous malformations involving the Galenic system. *AJR* 1970;**110**:50–5.

[3] Lasjaunias P, Ter-Brugge K, Lopez-Ibor L, *et al.* The role of dural anomalies in vein of Galen aneurysms: report of six cases and review of the literature. *AJNR* 1987;**8**:185–92.

[4] Hoffman HJ, Chuang S, Hendrick EB, *et al.* Aneurysm of the vein of Galen. Experience at the Hospital For Sick Children, Toronto. *J Neurosurg* 1982;**57**:316–22.

[5] Padget DH. Cranial venous system in man in reference to development, adult configuration and relation to arteries. *Am J Anat* 1956;**98**:307–55.

[6] Amacher AL, Shillito J. The syndromes and surgical treatment of aneurysms of the great vein of Galen. *J Neurosurg* 1973;**39**: 89–98.

[7] Aleem A, Knesevich MA. Schizophrenia like psychosis associated with vein of Galen malformation. *Can J Psychiatry* 1987;**32**:226–7.

[8] Ventureyra EC, Badejo A. Galenic arteriovenous malformation with precocious puberty. *Surg Neurol* 1984;**2**:45–8.

[9] Johnson IH, Whittle IR, Besser M, *et al.* Vein of Galen malformation: diagnosis and management. *Neurosurgery* 1987; **20**:747–58.

[10] Vergesslich KA, Ponhold W, Wimmer M, *et al.* Diagnosis of an aneurysm of the great cerebral vein of Galen using duplex sonography. *Pediatr Radiol* 1986;**21**:377–84.

[11] Vintzileos AM, Eisenfeld LI, Campbell WA, *et al.* Prenatal ultrasonic diagnosis of arteriovenous malformation of the vein of Galen. *Am J Perinatol* 1986;**3**:209–11.

[12] Roosen N, Schirmer M, Lins E, *et al.* MRI of an aneurysm of the vein of Galen. *AJNR* 1986;**7**:733–5.

[13] Pun KK, Yu YL, Huang CY, *et al.* Ventriculo-peritoneal shunting of acute hydrocephalus in vein of Galen malformation. *Clin Exp Neurol* 1987;**23**:209–12.

[14] McCord FB, Shields MD, McNeil A, *et al.* Cerebral arteriovenous malformation in a neonate. Treatment by embolisation. *Arch Dis Child* 1987;**62**:1273–5.

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Original articles

Interferon alfa, infectious virus, and virus antigen secretion in respiratory syncytial virus infections of graded severity

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SUMMARY Interferon alfa was measured by an immunoradiometric assay in the nasopharyngeal secretions of a group of infants admitted to hospital with respiratory syncytial virus infection. Virus replication in the upper respiratory tract was assessed by infectivity assay and by an enzyme linked immunoadsorbent assay for the viral fusion protein on the same nasopharyngeal secretions. All infants were examined daily while in hospital and allocated a score based upon a subjective assessment of the severity of their illness. There was no significant correlation between interferon, virus, or fusion (F) protein secretion and severity of illness or age of infant. It is concluded that poor interferon alfa secretion does not underly the susceptibility of infants to severe infections with this virus.

A defect in the production of interferon has been associated with exacerbation of viral respiratory disease in children[1, 2] and in animal model systems.[3] Respiratory syncytial virus is a major cause of respiratory disease, particularly bronchiolitis, in infants and children. This virus, although sensitive to the action of exogenously applied interferon in tissue culture, is a poor inducer of interferon in human cells.[4, 5] Interferon concentrations in the nasal secretions of infants, children, and adults with respiratory syncytial virus infection are low when compared with those in the secretions of patients of similar age infected with influenza viruses.[6-9] This allows the suggestion that poor in vivo interferon production may contribute to the susceptibility of infants already compromised by the immaturity of their immune response during a primary respiratory syncytial virus infection. It is noteworthy that parainfluenza virus type 3, which is also associated with bronchiolitis in infants of this age, is also a poor inducer of interferon in vivo.[9]

In order to test this hypothesis we measured interferon alfa concentrations in the nasopharyngeal secretions of a group of infants with respiratory syncytial virus infection. The association between interferon secretion, the severity of illness, and the degree of virus replication in the upper respiratory tract has been explored.

Patients and methods

Fifty seven infants were studied during admission to hospital in Nottingham between January and March 1984. All were diagnosed as having acute viral bronchiolitis.[10] Respiratory syncytial virus antigens were shown in nasopharyngeal secretions at admission by immunofluorescence staining of cells in 54 and in all by an enzyme linked immunoadsorbent assay (ELISA) for viral fusion (F) protein (see below). There were 30 boys and 27 girls with a mean (SD) age of 15·3 (9·4) weeks, range 3–40 weeks. Prodromal symptoms had begun from a mean of 4·2 (2·5) days, range 1–14 days, before admission. One child was still being exclusively breast fed at 12 weeks, and 20 others had been breast fed for a mean of 5·3 (5·1) weeks, range 0·5 and 20 weeks. There was a first degree family history of asthma and eczema or hay fever, or both in 21 infants; 38 mothers and 34 fathers were smokers and one or other parent smoked in 46 cases.

While in hospital each patient was examined daily by one of us (MSCW); the clinical variables cough, rhonchi, crepitations, subcostal recession, respiratory distress, and an empirical 'end of bed assessment' were allocated scores of 1–4 for minimal, mild, moderate, or severe respectively and the worst daily score (highest) for each variable noted at the time of discharge. Each child was reviewed three weeks after discharge and the number of days to full recovery recorded. To the above worst daily scores were added scores of 1–4 for hospital stay (1–2, 3–4, 5–6, 7 or more days) and total length of illness (prodrome+hospital+days to recovery 1–4, 5–8, 9–12, 13 or more days) and the resultant total score (range 8–32) was divided by the number of variables (8) to give a final illness severity score (range 1–4). These final severity scores were used for statistical analysis against virological variables described below.

Nasopharyngeal secretions were collected daily from all infants and flushed into a standard volume of virus transport medium containing 0·3 μmol/l phenolsulphonphthalein (phenol red), snap frozen in liquid nitrogen and stored at −70°C. The volume of secretion was calculated from the degree of dilution of the phenolsulphonphthalein in transport medium after the sample had been added as described previously.[9] Samples of nasal secretion below 30 μl could not be measured accurately and were excluded from the analysis.

ASSAY OF INFECTIOUS VIRUS AND INTERFERON IN SECRETIONS

Thawed nasal secretions were diluted in twofold steps in cell maintenance medium and assayed for the presence of infectious virus on microtray monolayers of HeLa cells. At three days postinoculation the monolayers were stained with a pool of subgroup cross reactive antirespiratory syncytial virus monoclonal antibodies[11] and foci of infection were visualised by the immunofluorescence method described by Routledge *et al.*[12] In parallel an aliquot of secretion was sonicated to disperse mucus and assayed for interferon alfa using an immunoradiometric kit as described by Taylor *et al.*[9]

ASSAY OF F PROTEIN IN SECRETIONS

Aliquots of secretion were diluted in 1% bovine plasma albumin, homogenised by sonication and dialysed against distilled water. Each specimen was concentrated approximately fivefold by freeze drying and resuspension in phosphate buffered saline. Respiratory syncytial virus F glycoprotein in the concentrated specimens was assayed by a double antibody sandwich ELISA.[13] The primary solid phase antibody layer was a pool of two monoclonal antibodies, 1A12 and 4E5, specific for separate epitopes on the F protein of respiratory syncytial virus and cross reactive with both subgroups of virus.[14] The detecting antibody combination was a polyclonal rabbit antiserum raised against the Long strain of respiratory syncytial virus as described by Gardner and McQuillin[15] followed by peroxidase conjugated swine antirabbit IgG (Dako Ltd). Specimens were tested in parallel with a standard pool of known positive secretions and appropriate corrections made for plate to plate variation. Titres were read from a standard curve constructed from seven replicate titrations of this standard pool.

STATISTICAL ANALYSIS

Infectivity, interferon, and antigen titres in original secretions were calculated after determination of secretion volume by the phenolsulphonphthalein dilution method. The sensitivity of recovery for each variable depended upon the sensitivity of the assay and the quantity of secretion recovered, which varied from patient to patient. The sensitivity of infectivity, interferon, and F antigen detection was taken as 2·6 $\log_{10}$ plaque forming units/ml, 1·28 $\log_{10}$ units/ml, and 1·5 $\log_{10}$ units/ml of secretion respectively. For statistical analysis, where assays were negative or titres/ml fell below these values, the titre/ml for the specimen was taken as equivalent to the sensitivity of detection.

Summated yields of infectivity, interferon, and F antigen over the first three days after admission were calculated from the formula:

$$\text{Summated titre} = \frac{x_1/\bar{x}_1 + x_2/\bar{x}_2 \ldots\ldots + x_n/\bar{x}_n}{n}$$

where $x_{1 \text{ to } n}$=titre on day 1 to day n and $\bar{x}_{1 \text{ to } n}$= mean titre for specimens for all patients tested on that day. Expression of each value as a fraction of the mean in this way allowed summation where samples from one of the three days was missing. Pairs of coordinates were analysed by product moment and Spearman's rank correlation.

Results

Severity scores were normally distributed around a mean (SD) of 2·55 (0·62). There was no correlation between the age of the infants and the severity of infection.

Measurable quantities of secretion were obtained on at least one of the first three days after admission for 53 of the 54 cases studied. Virus was demonstrable from 30 (57%) of these patients. Mean infectivity titres were highest for the first sample tested falling thereafter so that after three days only two of 32 tested were positive (figure A). Infectivity in the secretions of five infants was followed throughout

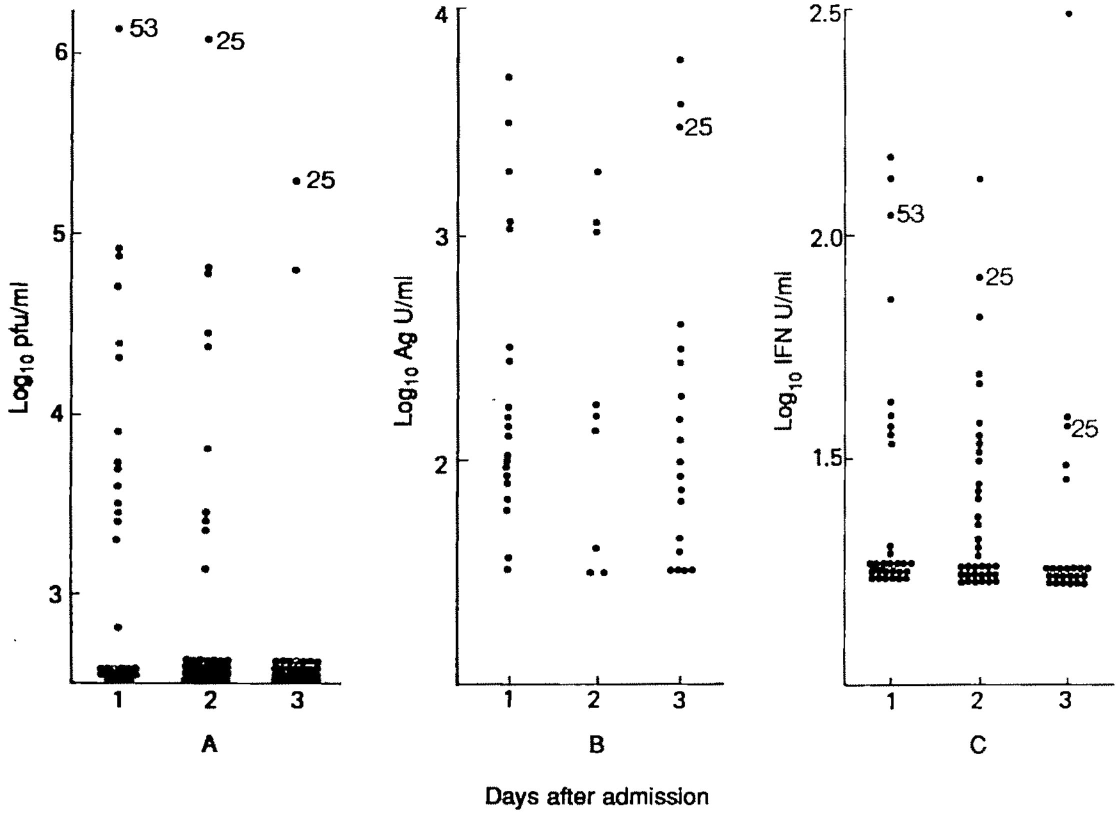

Figure *A: infectious virus (log_{10} plaque forming units (pfu)/ml), B: viral F protein (log_{10} Ag U/ml), and C: interferon alfa (log_{10} IFN U/ml) recovered from the nasopharyngeal secretions of infants admitted to hospital with respiratory syncytial virus infection. Each point represents one patient and the points representing patients 25 and 53 are singled out.*

their stay in hospital. None showed a rise in virus titre after the third day.

In a preliminary experiment F protein was assayed in daily nasopharyngeal secretions from six patients throughout their stay in hospital. Mean levels recovered, uncorrected for volume, were similar for three days postinfection but then declined, although four of the six patients still secreted detectable F protein when tested one week after admission. Thirty six patients were tested by the same technique during the first three days after admission. All but two were positive on at least one day. Mean concentrations of F protein/ml of secretion remained essentially constant over three days (figure B). There was a highly significant positive correlation between summated F protein and infectivity titres (table).

Table *Correlations of infectious virus, interferon, and F protein concentrations in nasopharyngeal secretions with age and severity of infection*

	Severity	*Infectivity**	*Interferon**	*F protein**
Age	−0·06	−0·26	−0·11	−0·15
Severity		+0·18	+0·1	+0·22
Infectivity			(+0·27)	(+0·47)**
Interferon				(+0·23)

*Summated values for the first three days after admission.
**$p<0·01$.
Figures in parentheses represent Spearman rank coefficients of correlation.

Although interferon was detectable in all but eight of the 54 infants tested only 26 secreted more than or equal to 1·28 $\log_{10}$ units/ml (19 units/ml), the baseline for inclusion as positive in statistical analyses. Mean interferon concentrations were similar on the first two days after admission. On the third day, however, there was a sharp decline in the number of infants with detectable interferon in their secretions (figure C). Four infants were followed for four to nine days. Although interferon was detectable in secretions of three of the four beyond three days and one remained positive until day eight, concentrations were low or unquantifiable because of the small volumes of secretion recoverable. There was a positive correlation between recovery of interferon with infectious virus and F protein but neither was significant (table).

Recovery of infectious virus, F protein, and interferon were negatively correlated with the age of the infants but positively correlated with the clinical severity of infection. In no case, however, did correlations approach significance. It is noteworthy that two infants, 53 and 25 (figure), secreted very high titres of infectious virus together with high concentrations of F protein and interferon. Both were less than 7 weeks old and were among the most severely ill in this study (severity scores 4·0 and 3·38 respectively).

Discussion

It is generally recognised that infants under 1 year of age are most vulnerable to respiratory syncytial virus, that the incidence of bronchiolitis peaks in the second or third month of life and that infants under 1 month of age generally are less severely affected.[16] Despite this appreciable age related variability in the pathogenesis of infection, in the group of infants studied here there was no correlation between age and clinical severity score. This bears out a previous study on a smaller group of patients[17] and probably relates to the relative uniformity of the group studied. All of the patients were between 3 and 40 weeks of age and all had been admitted to hospital with respiratory disease later diagnosed as respiratory syncytial virus infection.

The uniformity of the group may also explain why no significant correlation was observed between virus secretion and the age of the infants. Hall *et al*.[18 19] while finding lower secretion of virus in infants under 1 month of age, found no such correlation in a group ranging from 3 weeks to 21 months. Interferon secretion has also been shown previously to be independent of age in such patients.[7] Within this relatively homogeneous group we have sought evidence that poor interferon production correlates with defective limitation of virus replication.

Virus replication was monitored in secretions after storage at −70°C. As the virus is highly labile to freezing and thawing, storage in this way reduces virus titres but patterns of secretion remain discernible as all the samples were treated in the same way. As expected, recoveries of virus were relatively poor with only half of the infants yielding detectable infectious virus, although all were positive for virus antigen. Infectivity titres fell considerably over the first three days after admission. In a study by Hall *et al* mean virus titres in nasal secretions did not fall significantly for five days.[18] In that study, however, the authors indicated that titres from samples taken early after admission may have been underestimated. For two young, severely ill individuals virus secretion was exceptionally high. Other infants who scored highly in the clinical assessment of severity, however, secreted only modest concentrations of virus and overall there was no correlation of virus secretion and severity of disease.

Secretions were also titrated for viral F protein by ELISA. The F protein antigen is relatively stable and is unaffected by prolonged periods of storage (data not shown). Although F protein secretion was strongly correlated with yields of infectious virus, secretion patterns differed. All of the infants tested were positive for F protein and levels of secretion were much more stable over the period of study. Furthermore, log summated values for F protein secretion were normally distributed.

Interferon secretion showed no significant correlation with clinical assessment of the severity of infection and was positively correlated with both virus infectivity and F protein antigen secretion whereas our hypothesis predicted a negative correlation. This pattern is similar to that found in other primary virus infections of the respiratory tract,[20 21] and this suggests that limitations in the secretion of alfa interferons, at least of those species detectable by the YOK-1 monoclonal antibody, do not underly the development of bronchiolitis. That neither interferon, infectivity, nor F protein secretion were significantly associated with clinical severity assessment also diminishes hope that such easily measured variables might assist in prognosis.

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References

[1] Isaacs D, Clarke JR, Tyrrell DAJ, Webster JR, Valman HB. Deficient production of leukocyte interferon (interferon alpha) in vitro and in vivo in children with recurrent respiratory tract infections. *Lancet* 1981;ii:950–2.

[2] Juhlin L, Cantell K. Interferon response of lymphocytes and resistance to infections. *Lancet* 1978;i:667–8.

[3] Hoshino A, Hiroshi T, Osamu M, Imanishi J, Kishida T, Tovey MG. Effect of anti-interferon serum on influenza virus infection in mice. *Antiviral Res* 1983;3:59–65.

[4] Gardner PS, McGuckin R, Beale AJ, Fernandes R. Interferon and respiratory syncytial virus. *Lancet* 1970;i:574–5.

[5] Chonmaitree T, Roberts NJ, Douglas RG, Hall CB, Simons R. Interferon production by human mononuclear leukocytes:

differences between respiratory syncytial virus and influenza viruses. *Infect Immun* 1981;**32**:300–3.

[6] McIntosh K. Interferon in nasal secretions from infants with viral respiratory tract infections. *J Pediatr* 1978;**93**:33–6.

[7] Hall CB, Douglas RG, Simons RL, Geiman JM. Interferon production in children with respiratory syncytial, influenza and parainfluenza virus infections. *J Pediatr* 1978;**93**:28–32.

[8] Hall CB, Douglas RG, Simons RL. Interferon production in adults with respiratory syncytial virus infection. *Ann Intern Med* 1981;**94**:53–5.

[9] Taylor CE, Craft AW, Kernahan J, Reid MM, Toms GL. Nasal interferon responses in leukaemia. *Arch Dis Child* 1985;**60**:829–31.

[10] Henry RL, Milner AD, Stokes GM. Bronchiolitis. *Am J Dis Child* 1983;**137**:805–6.

[11] Routledge EG, McQuillin J, Samson ACR, Toms GL. The development of monoclonal antibodies to respiratory syncytial virus and their use in diagnosis by indirect immunofluorescence. *J Med Virol* 1985;**15**:305–20.

[12] Routledge EG, Willcocks MM, Samson ACR, Morgan L, Scott R, Toms GL. The purification of four respiratory syncytial virus proteins and their evaluation as protective agents against infection in Balb/c mice. *J Gen Virol* 1988;**69**:293–303.

[13] Voller A, Bidwell D, Bartlet A. Microplate enzyme immunoassay for the diagnosis of virus infection. In: Rose NR, Friedman H, eds. *Manual of clinical immunology*. Washington: American Society for Microbiology, 1976:506–12.

[14] Morgan LA, Routledge EG, Willcocks MM, Samson ACR, Scott R, Toms GL. Strain variation of respiratory syncytial virus. *J Gen Virol* 1987;**68**:2781–8.

[15] Gardner PS, McQuillin J. *Rapid virus diagnosis: application of immunofluorescence*. 2nd ed. London: Butterworth, 1980.

[16] Sims DG, Downham MAPS, McQuillin J, Gardner PS. Respiratory syncytial virus infection in North-East England. *Br Med J* 1976;**ii**:1095.

[17] Scott R, Pullan CR, Scott M, McQuillin J. Cell mediated immunity in respiratory syncytial virus disease. *J Med Virol* 1984;**13**:105–14.

[18] Hall CB, Douglas RG, Geiman JM. Quantitative shedding patterns of respiratory syncytial virus in infants. *J Infect Dis* 1975;**132**:151–6.

[19] Hall CB, Douglas RG, Geiman JM. Respiratory syncytial virus infections in infants: quantitation and duration of shedding. *J Pediatr* 1976;**89**:11–5.

[20] McLaren C, Potter CW. The relationship between interferon and virus virulence in influenza virus infection of the mouse. *J Med Microbiol* 1972;**6**:21–32.

[21] Husseini RH, Sweet C, Collie MH, Smith H. The relation of interferon and non-specific inhibitors to virus levels in nasal washes of ferrets infected with influenza viruses of different virulence. *Br J Exp Pathol* 1981;**62**:87–93.

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IgG and IgM antibodies to viral glycoproteins in respiratory syncytial virus infections of graded severity

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SUMMARY Serum antibodies to the fusion (F) and large glycoprotein (G) of respiratory syncytial virus in the serum of 57 infected infants were measured by enzyme linked immunosorbent assay (ELISA). Most serum samples taken at the time of admission to hospital contained antibodies to both glycoproteins, and overall there was no significant evidence of a selective deficiency of antibody to either viral antigen. Less than a quarter of the infants showed rising IgG antibody titres to either glycoprotein after infection, whereas over threequarters produced an IgM response. There was a significant correlation between IgG response to viral glycoproteins and the age of the infant. The correlation of age with the IgM response was less pronounced, and there was no correlation between serum IgG antibody derived transplacentally in the acute phase of infection and IgM response to either glycoprotein. Neither IgG or IgM responses correlated with a clinical assessment of the severity of infection in the infants. IgM responses, however, were weakly correlated with reduced secretion of infectious virus in the upper respiratory tract.

Bronchiolitis after respiratory syncytial virus infection is most prevalent in the first six months of life when concentrations of transplacentally acquired antibody to the virus in infant serum samples are high.[1] The beneficial effect of passively acquired maternal antibody is uncertain. In animals, passive administration of antibody before infection is protective[2] and, in preliminary experimental studies in human infants, treatment with immune globulin has proved to be beneficial.[3] Commensurate with these findings bronchiolitis is rare in the first month of life when antibody titres acquired transplacentally are high,[4] and epidemiological studies indicate that infants whose mothers have the highest concentrations of serum antibody at birth are protected for longer.[5 6]

Nevertheless, for infants admitted to hospital with bronchiolitis, maternal antibody must have been inadequate to control their infection, although it is often present at reasonable concentrations. In these infants serum IgG antibody responses to the virus are low and frequently undetectable,[7] although most infants mount a local IgA antibody response.[8] It is possible that IgG antibody in the serum acquired transplacentally merely masks the development of the infant IgG response but it has been suggested that maternal antibody may also be immunosuppressive.

Respiratory syncytial virus bears two glycoproteins upon its surface—the G glycoprotein that is responsible for virion attachment to susceptible cells and the F or fusion glycoprotein that mediates fusion of virion and cellular membranes and thus intracellular penetration of the virus.[9 10] Antibodies to both glycoproteins can neutralise the virus and immunisation with these (but not internal virion proteins) is protective in animals.[11] The F glycoprotein seems to generate more effective immunogenic responses. Antigenic variation in the G glycoprotein has been reported between isolates of respiratory syncytial virus, although the F glycoprotein seems remarkably stable. This suggests that in humans the G protein is an important protective antigen and subject to selective pressure by the immune response.[12 13] Murphy *et al* have reported that the immunosuppressive effect of antibody acquired transplacentally is most pronounced on the antibody response to the G glycoprotein of the virus.[14] In other studies anti-G antibody concentrations have been found to be low or absent in serum samples from infants with bronchiolitis, and it has been suggested that susceptibility to the virus may result from an inability to respond to the G glycoprotein.[15 16]

We have recently purified the two glycoproteins of a subgroup A strain of respiratory syncytial virus.[11] In the present study we have used immunoglobulin class specific enzyme linked immunosorbent assays (ELISA) for antibodies to these proteins to compare the IgG and IgM antibody responses to G and F glycoproteins in a group of infants admitted to hospital with respiratory syncytial virus infection, and to assess the inhibitory effect of antibody acquired transplacentally on these responses. In addition we have investigated correlations between antibody responses, viral titres in the upper respiratory tract, and the clinical severity of infection to assess the importance of each glycoprotein specific antibody in protection.

Patients and methods

Fifty seven infants between 3 and 40 weeks of age admitted to the Queen's Medical Centre, Nottingham between January and March 1984 with respiratory syncytial virus infection were subjected to intensive clinical and virological assessment described in detail elsewhere (C E Taylor, unpublished observations). Briefly, infection was diagnosed by immunofluorescence, severity of infection was assessed by daily examination resulting in a severity score at discharge from hospital, and nasopharyngeal secretions were taken daily and titrated for infectious virus by an infectious focus assay.

Blood samples were collected by venepuncture on admission and about 10 weeks later (mean (SD) 67 (17) days), frozen immediately in liquid nitrogen, and stored at −70°C until thawed for assay.

ELISA FOR ANTIBODIES TO RESPIRATORY SYNCYTIAL VIRUS GLYCOPROTEINS F AND G

Immunoaffinity purified respiratory syncytial virus fusion glycoprotein (F) and large glycoprotein (G) were prepared as previously described.[11] Samples of human serum were titrated for antibody against each protein by ELISA as described by Voller *et al.*[17] Briefly, Dynatech 129A microtitre plates were coated overnight with 33 μg/ml of F, or 60 μg/ml of G, glycoprotein in bicarbonate buffer at pH 9·6. After washing in phosphate buffered saline containing 0·5% Tween 20 (Sigma) the plates were loaded with 50 μl/well of serum diluent and each sample was diluted in fourfold steps. For IgM assays a protein extract of HeLa cells prepared as described by Nandapalan *et al* was included in the conjugate diluent at a final concentration of 10% to reduce non-specific binding.[18] After incubation for 90 minutes at 37°C to allow adsorption, the binding of IgG was detected with peroxidase conjugated rabbit immunoglobulins to human IgG (γ chains) or to human IgM (μ chains), as appropriate. The binding of conjugate was determined by standard methods using orthophenylene diamine as a substrate.

Titres for samples were determined by comparison with a standard serum pool in each plate. Differences in titre between test and standard serum assays were determined on the straight line portion of their curves and the difference in titre was added to the known titre of the standard serum previously determined as the mean of the reciprocal of the dilution giving an optical density of 1·0 in repeated assays. An optical density of 0·2 above serum free, capture plus conjugate, control wells was arbitrarily taken as the cut off between positivity and negativity in all assays. As the sensitivity of assays for different immunoglobulins varied the cut off corresponded to a different minimum detectable titre in each case. In the 5–10 plates used for each assay type the cut off corresponded to a $\log_{10}$ titre of 1·1 (0·5), 1·6 (0·7), 0·8 (0·4), and 0·76 (0·4) for IgG anti-F and G glycoprotein, and IgM anti-F and G glycoprotein assays, respectively. For statistical analyses negative results were taken as 0·1 below the $\log_{10}$ cut off value for the given plate. In each plate an aliquot of a second serum pool was titrated to give an estimate of the reproducibility of the assay. In over 10 assays of each type the results for this serum were mean (SD) $\log_{10}$ 3·0 (0·3), 2·9 (0·23), 1·5 (0·36), and 1·82 (0·32) for IgG anti-F and G glycoprotein, and IgM anti-F and G glycoprotein assays, respectively.

As a check on specificity ascites fluids containing mouse monoclonal antibodies to the F and G glycoproteins and to the viral nucleoprotein, matrix protein, phosphoprotein, and 22K protein, were titrated on F and G glycoprotein coated plates using a rabbit antimouse immunoglobulin peroxidase conjugate (ICN Biomedicals). With the F glycoprotein as antigen, the anti-F glycoprotein monoclonal antibody gave a titre of >1/640 000, the anti-22K protein monoclonal antibody gave a titre of 1/199, and all the others were negative at 1/100. With the G glycoprotein as antigen, and anti-G monoclonal antibody gave a titre of 1/640 000 but all the others were negative at 1/100.

ASSAY FOR RHEUMATOID FACTOR

Rheumatoid factor in serum samples was estimated in an assay similar to that for specific IgM antibody except that the plates were coated with 10 μg/ml of affinity purified human IgG instead of respiratory syncytial virus glycoprotein, and samples were diluted in diluent that did not contain HeLa cell extract. Titres were calculated in the same way as for specific antibody using the same serum standards which both contained low levels of rheumatoid factor. The assay was standardised by titration of five positive control serum samples. The effective limit of detection of the test was 0·14 IU of rheumatoid factor in 16 µl of serum. The mean (SD) reproducibility of 12 independent assays made on frozen aliquots of a known postive serum was 1·78 (0·54) IU/ml.

STATISTICAL ANALYSIS

When variables were normally distributed comparisons were made and correlation coefficients derived by least squares linear regression. Where one of the variables was not normally distributed and correlations seemed significant the variables were also tested by Spearman's rank test.

Results

More infants possessed IgG antibody to the F protein than to the G protein at the acute stage of infection (table 1). Mean titres of antibody to the F protein were slightly higher than those to the G protein. Although overall there was no significant rise in mean antibody to either protein between acute and convalescent samples, individuals did show rises not accountable for by assay variability. The log increment in IgG antibody to both F and G glycoproteins was normally distributed. Twice as many infants mounted a rise in antibody against the F protein as against G. This difference was not significant (χ^2 test).

IgM antibodies to both proteins were detected in the serum samples of most infants at the time of admission to hospital. In only a few was there a further increase on testing two to three months later. This is consistent with the transient nature of the IgM response, peak titres being expected at three to four weeks after infection.[19] As a check on the specificity of the assay, serum samples were collected from five normal infants at seven weeks postpartum with no history of respiratory syncytial virus infection. All five possessed serum IgG antibodies to both glycoproteins ranging in titre from 2·4 to 3·0 log$_{10}$ but none possessed detectable IgM antibodies. Although there was evidence of rheumatoid factor in 48 of the 86 samples from infected infants tested, in only five were levels as high as half of the specific antibody titre. These samples were omitted from the analysis. The log of the remaining IgM antibody titres to both F and G glycoproteins was normally distributed. No differences in IgM response to the two proteins were discernible.

IgG antibodies to the virus at the acute stage of infection may largely consist of maternal antibody acquired transplacentally that declines in titre with postpartum age. This conclusion is supported by the significant negative correlations between the age of the infant and the IgG antibody titres to both proteins at the acute stage of infection (table 2). A rise in antibody between acute and convalescent samples is a measure of the infant's own immune response. Significant positive correlations between age and

Table 2 *Correlation coefficients between age and class specific antibody responses to respiratory syncytial virus glycoproteins*

Antigen	*Immunoglobulin class*		
	IgG		*IgM*
	Acute	*Rise*	*Acute*
F	−0·6**	+0·5**	+0·19
G	−0·4*	+0·3*	+0·4*

*=p<0·05; **p=<0·01.

Table 1 *Antibody responses to the F and G glycoproteins of respiratory syncytial virus*

Antibody class	*Antigen*	*Acute*		*Convalescent*		*No (%)* of significant responses*	*Mean rise (log$_{10}$)*
		No (%) antibody positive	*Mean (SD) titre (log$_{10}$)*	*No (%) antibody positive*	*Mean (SD) titre (log$_{10}$)*		
IgG	F	46/50 (92)	2·34 (0·09)	43/45 (96)	2·44 (0·08)	10/43 (23)	+0·1
	G	40/54 (74)	2·13 (0·08)	31/45 (69)	2·04 (0·09)	5/44 (11)	−0·09
IgM	F	42/51 (82)	1·31 (0·01)	36/40 (90)	1·33 (0·1)	2/44 (5)	+0·02
	G	38/52 (73)	1·16 (0·06)	45/45 (100)	1·35 (0·08)	3/44 (7)	+0·19

*A rise of equal to or greater than twice the standard deviation for repeated determinations of a single serum sample was taken as significant. Thus rises of 0·6, 0·72, 0·72, and 0·74 log$_{10}$ or above were taken as significant for IgG anti-F, IgG anti-G, IgM anti-F, and IgM anti-G, respectively.

Table 3 *Correlation coefficients between titre of infectious virus and antibody response to respiratory syncytial virus glycoproteins*

Antigen	*Immunoglobulin class*		
	IgG		*IgM*
	Acute	*Rise*	*Acute*
F	+0·14	−0·30	−0·32 (−0·20)†
G	−0·02	−0·04	−0·36* (−0·17)†

$^*p<0\cdot05$.

†Figures in parentheses represent Spearman's rank correlation coefficient.

rises in antibody after infection suggest that older infants are able to respond more effectively. This conclusion is underlined by positive correlations between age and titres of IgM antibodies in acute sera. IgM antibodies do not cross the placenta and their presence in samples taken during the acute stage indicate an early response by the infant. The stronger correlation of age with the IgM response to the G protein may indicate that younger infants find it difficult to respond to this protein.

An increase in responsiveness with age may result from an inhibitory effect of antibody acquired transplacentally on the infants immune response. The ability of infants to make an IgM antibody response was not, however, correlated with acute serum titres of IgG antibody to either glycoprotein ($r=0\cdot04$ in both cases).

There were no significant correlations between the severity of disease and titres of antibody in acute or convalescent samples to either glycoprotein or of either immunoglobulin class or rises in the titre of such antibodies after infection. There was, however, a general tendency for evidence of an immune response judged by rising titres of IgG antibody or high IgM antibody in samples taken during the acute phase to correlate with secretion of lower titres of infectious virus (table 3). Log infectious virus yields were not normally distributed, and this tendency was not significant when a non-parametric analysis was applied.

Discussion

A central question in this study is whether infants encountering respiratory syncytial virus for the first time at an early age develop a severe lower respiratory tract infection because of a selective deficiency of antibody to one of the virion glycoproteins as suggested by Hildreth *et al.*[16] They reported lower titres of ELISA anti-G antibodies in serum samples from cord blood of infants who were infected with the virus in their first year compared with uninfected controls. These findings conflict with those of a previous study in which higher titres of antibody to the viral nucleoprotein but not the G glycoprotein were found in the serum samples of mothers whose babies subsequently escaped infection.[15] It remains unclear whether the protective effect of maternal antibody measured at birth in these experiments is exerted directly upon the infant or indirectly by protecting the mother and thus reducing the risk of viral transmission to the infant in the first month of life. In this study we could find no correlation between titre of IgG antibody to either glycoprotein in serum samples taken during the acute phase, a measure of residual antibody that had been acquired transplacentally, and the clinical severity of infection. Neither was there any significant correlation between such antibody and virus titres in the respiratory tract. This suggests that antibodies acquired transplacentally have little direct influence on the course of infection in the infant, favouring the hypothesis that maternal antibodies protect the infant indirectly.

Ward *et al* observed that after infection anti-G, but not anti-F, responses were all but absent in younger infants.[15] Subsequent studies employing ELISA against purified virion glycoproteins confirmed that both serum and mucosal IgG and IgA responses were poor in younger infants but there were no significant differences between anti-G and anti-F responses.[14] In the present study we also observed poor IgG responses to both glycoproteins after infection and although responses to the F protein were about twice as good as those to the G protein, this difference was not significant. Lower anti-G antibody titres might be expected if the infants were infected with a subgroup B virus, as the glycoproteins used in the assays were derived from a subgroup A virus and there is a wider divergence of antigenicity between the G than the F proteins of the two subgroups.[19] Unfortunately no means of subgrouping viruses was available when the specimens were collected.

As measurement of IgG responses is complicated by the presence of residual maternal antibody, the infants' immune responses to the two glycoproteins were further compared by measuring the infant IgM antibody response, the measurement of which is untrammelled by passively acquired IgG. Serum samples from over three quarters of the infants tested contained IgM antibodies on admission to hospital. This rate of response is consistent with that previously reported using virus infected cells as antigen, although in previous studies maximal responses have been detected a week after the onset of illness.[19 20] IgM antibody titres to both glycoproteins in samples taken during the acute phase were similar, and there was no evidence of a deficiency in antibody to the G glycoprotein. Furthermore, no correlations between IgM antibody responses to either glycoprotein and the severity of illness were found. Serum IgM responses, but not IgG responses, were negatively correlated with virus in secretions. The correlation was weak and not significant when a non-parametric test was applied. This does, however, parallel the correlation between the local IgA response and cessation of virus shedding reported by McIntosh *et al*[8] and confirms the importance of the early antibody responses in terminating virus secretion.

The infant antibody response was significantly greater in older infants, but improvement with age was more pronounced with IgG than with IgM. Murphy *et al* studied serum IgA responses and found that anti-F glycoprotein responses were related to age alone, while anti-G glycoprotein responses were determined by residual titres of maternal antibody that decayed with age.[14] This does not seem to be so for the IgM response as, here, titres of IgM antibody to neither glycoprotein showed any significant negative correlation with maternal antibody in samples taken during the acute phase. This result is encouraging as it suggests that vaccination of young infants may be able to stimulate an IgM response despite the presence of high levels of maternal antibody in the serum. Such antibodies might offer some protection by restricting virus replication in the upper respiratory tract early in infection.

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References

[1] Parrot RH, Kim HW, Arrobio JO, *et al*. Epidemiology of respiratory syncytial virus infection in Washington, D.C. II. Infection and disease with respect to age, immunologic status, race and sex. *Am J Epidemiol* 1973;**98:**289–300.

[2] Taylor G, Stott EJ, Bew M, Fernie BF, Cote PJ. Monoclonal antibodies protect against respiratory syncytial virus. *Lancet* 1983;**ii:**976.

[3] Hemming VG, Rodriguez W, Kim HW, *et al*. Intravenous immunoglobulin treatment of respiratory syncytial virus infections in infants and young children. *Antimicrob Agents Chemother* 1987;**31:**1882–6.

[4] Neligan CA, Steiner HM, Gardner PS, McQuillin J. Respiratory syncytial virus infection of the newborn. *Br Med J* 1970;**ii:**146.

[5] Ogilvie MM, Vathenen AS, Radford M, Codd J, Key S. Maternal antibody and respiratory syncytial virus infection in infancy. *J Med Virol* 1981;**7:**263–71.

[6] Glezen WP, Paredes A, Allison JE, Taber, Frank AL. Risk of respiratory syncytial virus infection for infants from low-income families in relationship to age, sex, ethnic group, and maternal antibody level. *J Pediatr* 1981;**98:**708–15.

[7] Bruhn FW, Yeager AS. Respiratory syncytial virus in early infancy—circulating antibody and the severity of infection. *Am J Dis Child* 1977;**131:**145–8.

[8] McIntosh K, McQuillin J, Gardner PS. Cell-free and cell-bound antibody in nasal secretions from infants with respiratory syncytial virus infections. *Infect Immun* 1979;**23:**276–81.

[9] Walsh EE, Brandriss MW, Schlesinger JJ. Purification and characterisation of the respiratory syncytial virus fusion protein. *J Gen Virol* 1985;**66:**409–15.

[10] Levine S, Klaiber-Franco R, Paradiso PR. Demonstration that glycoprotein G is the attachment protein of respiratory syncytial virus. *J Gen Virol* 1987;**68:**2521–4.

[11] Routledge EG, Willcocks MM, Samson ACR, *et al*. The purification of four respiratory syncytial virus proteins and their evaluation as protective agents against experimental infection in BALB/c mice. *J Gen Virol* 1988;**69:**293–303.

[12] Morgan LA, Routledge EG, Willcocks MM, Samson ACR, Scott R, Toms GL. Strain variation of respiratory syncytial virus. *J Gen Virol* 1987;**68:**2781–8.

[13] Hendry RM, Fernie BF, Anderson LJ, McIntosh K. Antigenic and epidemiologic analysis of distinct strains of respiratory syncytial virus from two successive community outbreaks (1983–85). In: Mahy B, Kolakofsky D, eds. *The biology of negative strand viruses*. Amsterdam: Elsevier, 1987:397–403.

[14] Murphy BR, Alling DW, Snyder MH, *et al*. Effect of age and pre-existing antibody on serum antibody response of infants and children to the F and G glycoproteins during respiratory syncytial virus infection. *J Clin Microbiol* 1986;**24:**894–8.

[15] Ward KA, Lambden PR, Ogilvie MM, Watt PJ. Antibodies to respiratory syncytial virus polypeptides and their significance in human infection. *J Gen Virol* 1983;**64:**1867–76.

[16] Hildreth SW, Belshe RB, Paradiso PR, Smith DH. Correlation between respiratory syncytial virus (RSV) glycoprotein specific maternal antibodies and RSV disease of infants. *Pediatr Res* 1987;**21:**917.

[17] Voller A, Bidwell D, Barlett A. Microplate enzyme immunoassay for the diagnosis of viral infection. In: Rose NR, Friedman H, eds. *Manual of clinical immunology*. Washington: American Society for Microbiology, 1976:506–12.

[18] Nandapalan N, Routledge E, Toms GL. An enzyme linked immunosorbent assay for IgG and IgA antibodies to respiratory syncytial virus in low dilutions of human serum and secretions. *J Med Virol* 1984;**14:**285–94.

[19] Johnson PR, Spriggs MK, Olmsted RA, Collins PL. The glycoprotein of human respiratory syncytial viruses of subgroups A and B: extensive sequence divergence between antigenically related proteins. *Proc Natl Acad Sci USA* 1987;**84:**5625–9.

[20] Welliver RC, Kaul TJ, Putman TI, Sun M, Riddlesberger RN, Ogra PL. The antibody response to primary and secondary infection with respiratory syncytial virus: kinetics of class-specific responses. *J Pediatr* 1980;**96:**808–13.

[21] Hornsleth A, Friis B, Grauballe PC, Krasilnikof A. Detection by ELISA of IgA and IgM antibodies in serum in primary lower respiratory syncytial virus infection. *J Med Virol* 1984;**13:**149–61

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Indolent glioma: a cause of epilepsy

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SUMMARY We studied eight patients with indolent gliomas. In all eight cases, despite characteristic radiological features, there was a delay in diagnosis because of misinterpretation of the initial computed tomogram. These tumours are an important cause of epilepsy of childhood, and surgery may well alleviate the epilepsy and possibly achieve a long term 'cure'. Therefore it is important that a diagnosis is made early and the lesion completely excised.

Since the days of Penfield and Jasper[1] varying types of operation and different selection criteria for surgery of epileptic patients have been advocated with variable results. Falconer suggested that successful long term cessation of epilepsy depends on finding and removing focal pathology.[2]

Over the last 15 years we have seen a group of patients with sizable indolent gliomas at this hospital. These children have all presented a diagnostic problem to the clinicians who first managed them. All the children have a lengthy history and the tumours all show characteristic radiological features. These patients constitute a group whose epilepsy may be successfully alleviated by surgery. It is our impression that this pathological entity has not received the attention that it deserves. These children have been referred from all parts of the United Kingdom because of our interest in epilepsy surgery. The true incidence, however, may be rather higher than the number suggests because we suspect the diagnosis is frequently missed.

Case reports

CASE 1

An 8 year old boy presented at the age of 3·5 years, with focal epilepsy. He was seen by a paediatrician and a paediatric neurologist in his home town and London. Various diagnoses were made including 'atypical absences' and 'drop attacks' of unknown cause. His fits remained poorly controlled and were occurring at a rate of three to 15 per day when seen by us. A plain skull radiograph showed scalloping of the inner table of the skull at the level of the coronal suture, just to the left of the midline (fig 1). A computed tomogram showed a low density lesion in the frontal lobe adjacent to the scalloping (fig 2). A complete macroscopic excision of the lesion was performed. Intraoperative electrocorticography showed epileptic discharges associated with the lesion which ceased after excision. Histological diagnosis was that of a low grade glioma. During the early postoperative period he suffered one focal fit. When reviewed seven years later he was well, fit free, and attending a normal school.

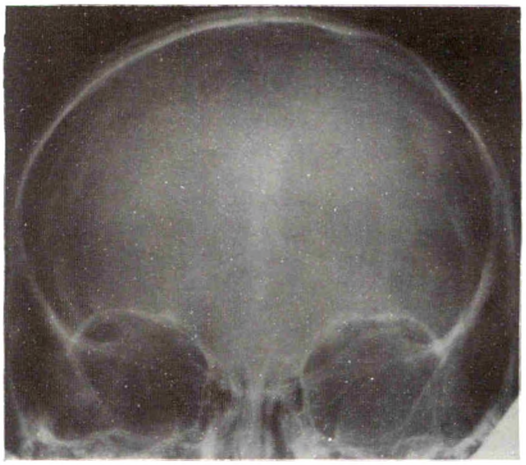

Fig 1 *Case 1: skull radiograph showing scalloping of the inner table of the skull at the level of the coronal suture just to the left of the midline.*

CASE 2

A 10 year old Asian girl living in Scotland first presented at the age of 3 with tingling in her left foot, sometimes associated with a twitching movement of her left leg. A wide variety of anticonvulsants

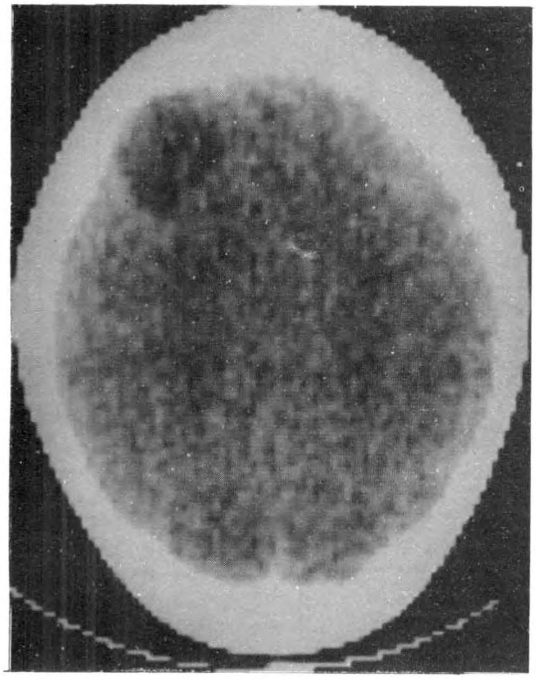

Fig 2 *Case 1: computed tomogram showing a low density lesion in the frontal lobe adjacent to the scalloping.*

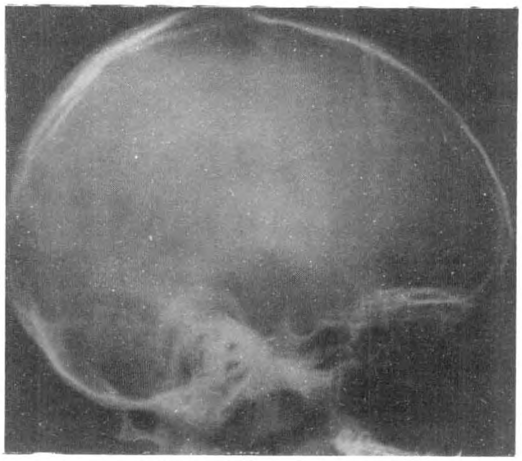

Fig 3 *Case 2: skull radiograph showing scalloping of the inner table of the skull.*

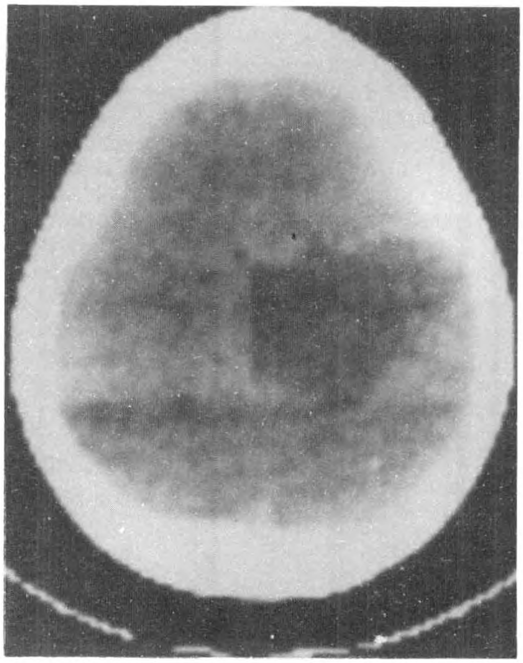

Fig 4 *Case 2: computed tomogram showing a low density lesion. Scalloping was not apparent in the computed tomogram, which was of less than ideal quality.*

including phenobarbitone, phenytoin, primadone, sodium valproate, and carbamazepine failed to control her attacks and she continued to have three to four fits per day. A skull radiograph was reported as normal and a computed tomogram showed a low density lesion in the right parietal lobe, thought to represent atrophy. Eventually she was referred to this hospital and on reviewing the skull radiographs and computed tomograms it was clear that this lesion was a space occupying lesion causing scalloping of the inner table of the skull (figs 3 and 4). At operation only a subtotal removal was possible as the lesion was situated in the central sulcus, extending to both motor and sensory cortex. Histology showed a protoplasmic astrocytoma. The patient was fit free for eight months before the attacks recurred. She had a full course of radiotherapy after which she had no seizures when reviewed four years later.

CASE 3

A 7 year old boy, born three months prematurely, first had right sided focal epilepsy at the age of 6. He subsequently had a grand mal attack. A computed tomogram at Oxford was reported to show a parasagittal glioma. He was then referred elsewhere to a paediatric neurosurgeon who regarded the lesion as atrophic. His epilepsy was poorly controlled with four to six attacks a day. He was eventually referred to this hospital where a subtotal removal of a tumour was carried out and histology showed a grade 2 astrocytoma. Postoperative recovery was uneventful apart from slight residual weakness on the right side. He was fit free for the first six weeks after surgery but has subsequently had approximately one focal fit per month.

CASE 4

A 6 year old girl, born eight weeks prematurely, presented at the age of 3 years with psychomotor epilepsy. This responded initially to carbamazepine but later failed to be controlled by phenytoin, sodium valproate, and carbamazepine. Her electroencephalogram showed gross abnormalities with frequent paroxysmal discharges and a possible right sided focus. She had a computed tomogram when she was 4 years old, which showed an area of low density in the right frontal pole. This was thought, by a paediatric neurologist, to be due to extensive scarring, possibly due to old haemorrhage. She was referred to this hospital and a repeat computed tomogram showed scalloping of the inner table of the skull adjacent to the low density lesion in the right frontal lobe (fig 5). A frontal lobectomy was performed and the histology showed a low grade glioma. She was entirely free of epilepsy when seen seven months after her operation.

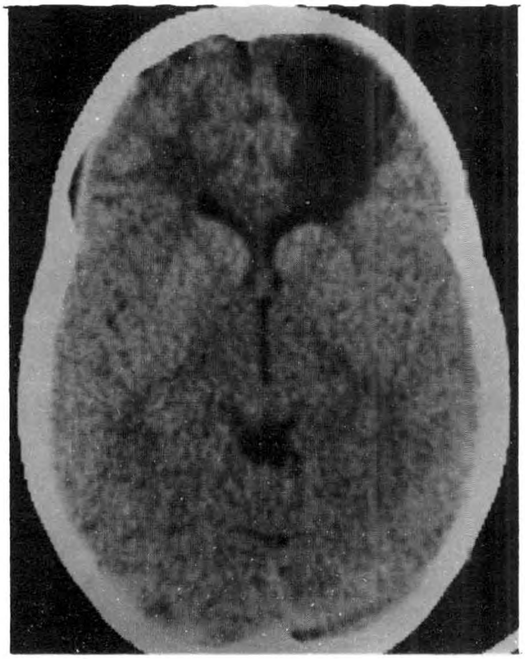

Fig 5 *Case 4: computed tomogram showing scalloping of the inner table of the skull adjacent to a low density lesion in the right frontal lobe.*

CASE 5

This boy first presented at the age of 3 years with absence attacks. By the age of 8 he began to have left sided focal epilepsy and by the age of 10 he had become aggressive and difficult to manage. He had been in trouble with the police and had been to a reform school. He was seen by various paediatricians and a paediatric neurologist. When seen by us at the age of 11 he was having one to three focal fits together with, on average, one grand mal fit per week. A plain skull radiograph showed thinning of the inner table of the skull in the left frontoparietal area. A computed tomogram showed a low density area just above the sylvian fissure extending fairly deeply half way between the vault and the falx cerebri. Complete macroscopic excision of the tumour was performed. Histology showed a grade 2 astrocytoma. Six years later he had had no further grand mal or focal epilepsy and was attending a normal school. His behaviour was much improved.

Since the writing of this paper we have had three more patients who had indolent gliomas presenting with epilepsy. The follow up after surgery was not long enough for them to be included in this series but they serve to illustrate further the clinical course and radiological features of indolent glioma.

CASE 6

An 8 year old boy first presented at the age of 5 with absence attacks and these developed into partial complex seizures. He was investigated with a computed tomogram on two occasions before being referred to us. Despite treatment he was having about 200 attacks per year. A repeat computed tomogram showed a high density lesion in the left temporoparietal lobe causing scalloping of the inner table. At operation the tumour was found to be straddling the sylvian fissure with vessels entering and leaving it. A total excision was therefore impossible. A biopsy of the tumour was carried out. Histology showed an oligodendroglioma. A left temporal lobectomy was also performed in the hope that this additional procedure would control his epilepsy. When reviewed three months after surgery he had had no fits.

CASE 7

A 13 year old boy presented at the age of 9 with an absence attack. Over the following year he developed grand mal epilepsy. A computed tomogram performed one year before admission showed a well defined area of a lower attenuation over the right parietal area with a small area of high density just superficial to it. This was thought to be the result of a previous haemorrhage from an underlying arter-

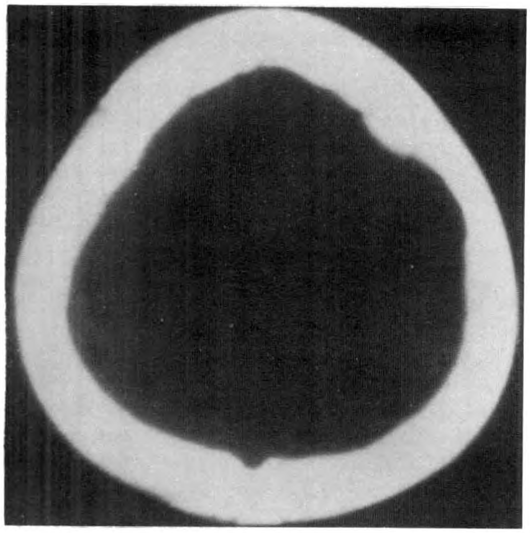

Fig 6 *Case 7: computed tomogram set at the bone window which showed scalloping of the inner table of the skull adjacent to a tumour.*

iovenous malformation. His fits were difficult to control and he had 63 fits during the week before admission. The computed tomogram performed at this hospital showed an indentation of the inner table of the skull just adjacent to the lesion (fig 6). Complete macroscopic excision of the lesion was performed and the histology was a low grade glioma. When seen six weeks after the operation he had had no further epilepsy.

CASE 8

A 13 year old left handed boy presented with epilepsy at the age of 8. Over the previous five years he had had increasing fits, up to six times a day. His parents noticed that his writing and feeding with his left hand had become increasingly poor. Within the last one year he became progressively more aggressive and disobedient and somewhat disinhibited. At one time the possibility of pseudoseizure was raised because there was no abnormality shown on electroencephalography. The patient was referred for psychotherapy without improvement. He had a computed tomogram performed in another hospital, which showed a lesion over right posterior frontal region and this was considered inoperable. A repeat scan was performed at this hospital. This showed that the tumour was more localised than suggested by the scan done previously and it indented the inner table of the skull. At operation subtotal removal of the tumour was performed. During the early postoperative period he had two episodes of focal fits but remained fit free when reviewed three months later. His parents reported dramatic improvement in the patient's behaviour and that 'he is a new person'.

The details of the eight patients are summarised in the table.

Discussion

The striking characteristic of this group is the delay in diagnosis that resulted from the misinterpretation of the initial findings on computed tomography. In some cases the diagnosis of epilepsy proved difficult and alternative diagnosis of 'pseudoseizures', etc, were commonly made, which added to the distress

Table *Details of eight patients studied*

	Case No							
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Age of onset of first fit (years)	3·5	3	6	3	3	5	9	8
Age at surgery (years)	9	10	7	6	11	8	13	13
Delay in diagnosis (years)	4·5	7	1	3	7	3	4	5
Extent of excision	Complete	Subtotal	Subtotal	Complete	Complete	Biopsy and temporal lobectomy	Complete	Subtotal
Complication	Nil	Nil	Mild hemiparesis which improved	Nil	Nil	Nil	Nil	Nil
Follow up	7 years	7 years	3 years	6 months	6 years	3 months	6 weeks	3 months
Results	No fits	No fits	Fit once per month	No fits	No fits	No fits	No fits	No fits

In cases 1–5 the mean age of onset of first fit was 3–7 years, mean age at surgery 8·4 years, mean delay in diagnosis 4·7 years, and mean follow up 5 years.

of the patients and their parents. The area of low density on the computed tomogram was frequently incorrectly diagnosed as atrophic or ischaemic yet the indentation of the inner table of the skull—which obviously is characteristic of a long standing, localised, space occupying lesion on the surface of the brain adjacent to the skull—was overlooked or unappreciated. This failure perhaps reflects undue concentration on the 'brain scan' as opposed to the 'head scan'; adjustment of the scan setting to show bone details is a particularly good method for showing the distortion of the inner table of the skull (fig 6).[3 4]

These patients also illustrate two other important facts: first, even subtotal removal of the tumour may produce extremely satisfactory alleviation of epilepsy and is worthwhile. Too many surgeons fail to consider the role of excisional surgery in treating the epilepsy and only assess the patient on the basis of excising the tumour in the hope of eradicating it. Thus when faced with a probable glioma all too often the surgeon retreats into surgical nihilism. This denies the patient and the family the chance of improvement or elimination of epilepsy, which is usually the predominant or sole symptom. On occasions it is possible to eradicate these tumours and such a chance should not be missed. This series reaffirms that the surgical treatment of epilepsy, that has withstood the test of time, is the removal of focal pathology—albeit incompletely on occasions.

It is incumbent on those surgeons treating epilepsy to emphasise the pathological; Oslerian principles of this surgery. All too often among the medical professions, surgical treatment of epilepsy is viewed as complex, difficult, time consuming, unsuccessful, dangerous, and requiring much expensive equipment. This partly accounts for the delay in referral among the patients whom we see in Oxford. In practice the vast majority of patients do not require complex investigation and the treatment is often straightforward and based on sound pathological precepts just as for any other surgical endeavour. A few patients may require prolonged and complex investigation but most do not. Patients with epilepsy due to a focal pathology should not be denied the possibility of surgery and its benefits and should be assessed by a neurosurgeon early.[5]

Secondly, this group also shows that if the epilepsy ceases after surgery then the behaviour of the patient rapidly improves and the child returns to normal. This improvement in behaviour cannot be ascribed to withdrawal of anticonvulsants as it is immediate and occurs despite maintenance of the same anticonvulsant regime. Family life returns to normal—parents can go out together, siblings can bring friends home again, quite apart from the patient being more able to live a normal existence. Indeed the family is cured as well as the patient after this surgery.

Is it impossible to make a correct diagnosis at an early stage? At least some of our patients were inferred to have a tumour from birth. This is supported by the observation of a palpable lump on one patient's head noticed shortly after birth by the mother. It was disregarded by her doctors and was later shown to have been caused by the expanding glioma. Thus a careful, detailed history and recognition of typical radiological features are important in the early diagnosis of these low grade gliomas.

The age of onset of epilepsy in our patients (cases 1–5) ranged from 3 to 8 years with a mean age of 3·7 years. The average age at diagnosis was 8·4 years. The average delay between initial presentation and surgery was therefore 4·7 years. In 1969 Page *et al* studied 23 cases of childhood glioma who presented with epilepsy and were detected late.[6] The age of onset of seizures range from 3 months to 8 years and the delay in diagnosis ranged from five to 20 years. By then 90% of the patients had developed abnormal neurological signs. Pipemier reported on patients with low grade astrocytomas of whom 38·4% had symptoms for more than five years.[7] Goldring *et al* recently reported a mean interval of 11 years from the onset of seizure to surgery that was mostly due to misinterpretation of abnormal computed tomograms as showing a non-neoplastic lesion.[8] Thus the difficulties in making an early diagnosis are not confined to the United Kingdom.

We specially emphasise the importance of appreciating the seizure pattern. If the fit always begins in the same way regardless of how it may subsequently develop, it suggests a focal origin and focal pathology in the brain. Abnormalities on computed tomography were misinterpreted initially in four cases: two as focal atrophy, one as extensive scarring, and one as an arachnoid cyst. No radiological investigation was performed on one patient for five years. The most important feature, as we have already stressed, which suggests an indolent glioma rather than scarring or atrophy, is indentation of the vault adjacent to the tumour. This was present in seven of our eight cases. This may be visible on a plain skull radiograph as in three of our patients. Computed tomograms have also improved our ability not only to localise the tumour but also to detect calcification. Magnetic resonance imaging may have a role in further delineating the lesion in doubtful case, such as differentiating a tumour from atrophy or a cyst.[9]

The average duration of follow up was five years. It is important to follow up the patients for at least 12 months before one can evaluate the result of epilepsy surgery. Three of our cases had no further fits at review seven years later. One patient who was operated on seven months ago remains fit free. Another patient still has focal fits but with decreased frequency.

In this series there was no operative mortality. Pipemier reported two surgical deaths in 60 patients with low grade astrocytoma.[7] The prognosis of indolent gliomas is generally favourable, but the tumour may perhaps become more aggressive after many years of quiescence. Complete excision can possibly achieve a 'cure'—as in two of our patients who have been followed up for seven years without recurrence.

It is a pleasure to thank Dr B Bower, Dr J Lindsay, and Dr G Stores who referred these patients to us.

References

[1] Penfield W, Jasper HH. *Epilepsy and the functional anatomy of the human brain.* Boston: Little, Brown and Co, 1954.

[2] Falconer MA. Surgical and pathological aspects of temporal lobe epilepsy. *Proc R Soc Lond [Biol]* 1953;**46**:971–4.

[3] Thomas JE, Baker HL. Assessment of roentgenographic lucencies of the skull: a systematic approach. *Neurology* 1975;**25**:99–106.

[4] Osborn RE, Ley CE. Astrocytoma with calvarial erosion. *AJNR* 1986;**7**:178.

[5] Adams CBT. Temporal lobectomy in young people. In: Ross E, Chadwick D, Crawford R, eds. *Epilepsy in young people.* Chichester: John Wiley, 1986:103–11.

[6] Page LK, Lombroso CT, Matson DD. Childhood epilepsy and late detection of cerebral glioma. *J Neurosurg* 1969;**31**:253–61.

[7] Pipemier AM. Observation on the current treatment of low grade astrocytic tumours of the cerebral hemispheres. *J Neurosurg* 1987;**67**:177–81.

[8] Goldring S, Rich K, Picker S. Experience with gliomas in patients presenting with a chronic seizure disorder. *Clin Neurosurg* 1986;**33**:15–42.

[9] Sperling MR, Wilson G, Engel J, Babb TL, Phelps M, Bradley W. Magnetic resonance imaging in intractable partial epilepsy: correlative studies. *Ann Neurol* 1986;**20**:57–62.

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Diet and faecal flora in the newborn: breast milk and infant formula

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SUMMARY This study examined the faecal flora on days 4, 14, and 28 of 17 breast fed babies and 26 bottle fed babies receiving a modern infant formula based on demineralised whey. Generally among breast fed babies bifidobacteria and staphylococci were the predominant organisms, whereas in the formula fed babies the predominant organisms were enterococci, coliforms, and clostridia. Despite the extensive modification of cows' milk to make an infant formula resemble human breast milk, the results are very similar to those previously reported with unmodified cows' milk baby feeds. The exact dietary factor responsible for these microbiological differences is unclear and in succeeding papers we have looked at the effects of protein quality, in particular the content of whey proteins, casein, and lactoferrin.

For many years it was accepted that the faecal flora of a breast fed baby differed from that of a bottle fed one.[1] There are three reasons for re-examining this association between diet and faecal flora in the newborn. Firstly, many of these studies were performed using infant formulas that consisted of cows' milk with only minimal modification, whereas cows' milk is now extensively modified in the manufacture of an infant formula.[2] Secondly, breast milk differs from cows' milk and cows' milk based formula in so many ways it is impossible to know which dietary difference is responsible for the microbiological difference. Thirdly, some studies have not found these differences: the faecal flora of breast and bottle fed babies were similar.[3, 4] It was suggested that the antiseptic environment of modern obstetrics may have over-ridden the dietary effects.

For these reasons we have re-examined the association between diet and faecal flora. This first paper determines whether the faecal microflora of the breast fed baby differs from that of a bottle fed baby receiving a well defined modern infant formula and succeeding studies explore the effects of specific nutrients.

Subjects and methods

DIET AND BABIES (TABLES 1 AND 2)

The infant formulas most commonly used in early life are based on demineralised whey plus skimmed milk, a blend of vegetable and animal fats, vitamins,

Table 1 *Composition of feeds compared with unmodified cows' milk (per l)*

	Breast milk	*Modern whey formula*	*Unmodified cows' milk*
Protein g (nitrogen×6·38)	11	17	33
Total 'true' protein (%)	9 (100)	16 (100)	31 (100)
Caseins	2·5 (28)	6·4 (40)	24·5 (79)
Whey proteins	6·5 (72)	9·6 (60)	6·5 (21)
Phosphorus (mg)	150	340	950
Iron (mg)	0·76	8·00	0·50

Table 2 *Data of babies taking part in the study*

	Breast fed (n=17)	*Formula fed (n=26)*
Sex:		
Male	8	12
Female	9	14
Race:		
White	15	19
Asian	0	6
Afro-Caribbean	2	1
Born by caesarean section	1	10
Mean (SD) birth weight (g)	3330 (390)	3290 (530)
Mean (SD) weight gain day 1–day 28 (g)	770 (340)	810 (330)

and minerals including iron. We therefore used such a formula (Nan supplied by Nestlé) for one group of babies, the other group was breast fed. Allocation of the babies to the two dietary groups depended solely on the mother's wish to breast or bottle feed her baby and so was not random. The babies in the two groups were similar in mode of delivery, size, etc. All were well and none received antibiotics. Mothers of the breast fed babies did not receive antibiotics postnatally. Breast fed babies were allowed feeds of water between breast feeds and the use of dummies was not prohibited.

PROCEDURES

Both groups of babies were fed on demand. The formula was reconstituted by the project nurses until the babies were 14 days old. From day 14 to day 28 mothers reconstituted the powdered formula. Anthropometric measurements were made at days 1 and 28. Faeces were collected on days 4, 14, and 28.

MICROBIOLOGICAL METHODS

Faeces were collected in nappy liners, weighed and emulsified in a transport medium (BHI broth containing 10% glycerol and 0·03% sodium formaldehyde sulphoxalate). This was immediately frozen and stored at −20°C. All specimens were analysed within one month of collection. Eight 10 fold dilutions were made in transport medium, and 20 µl of each dilution were inoculated in triplicate on to MacConkey agar (for enterobacteriaceae, *Staphylococcus* spp, and *Enterococcus* spp), Rogosa agar (for *Lactobacillus* spp and *Bifidobacterium* spp), and Blood agar containing 0·01% neomycin (for *Clostridium, Bacteroides,* and *Bifidobacterium* spp). Bacteria were identified by standard methods outlined by Cowan.[5] API20E were used to identify enterobacteriaceae, API20A were used to identify anaerobes (API System SA). *Lactobacillus* spp were identified using API50CHL. *Bifidobacterium* spp were identified biochemically using API50CHL as media and comparing the profiles with those of reference species of bifidobacteria obtained from the National Institute of Research in Dairying, Shinfield and to the schemes drawn up by Holdeman, Cato, and Moore and Mitsuoka and Kaneuchi.[6 7]

FAECAL PH

The pH of each faeces sample was measured in a 10% suspension in normal saline. All measurements were taken in a single batch using a Bibby 3 in 1 stick meter with an SMP 1 electrode.

STATISTICAL ANALYSIS

The results were analysed statistically using Mann-Whitney, Wilcoxon, χ^2 (with Yates's correction), and McNemar (with Yates's correction) tests as appropriate.

ETHICAL APPROVAL

Approval for the study was obtained from the ethics committee of the South Birmingham Health Authority. The mothers of all babies taking part in the study gave written informed consent.

Results

MICROBIOLOGY

There is no accepted convention for presenting descriptions of faecal flora. We found difficulty in summarising the data (for example, as means and SDs, etc) without covering up details of the distribution. We have therefore chosen to present the results in two ways: (1) counts of individual *organisms* in each baby shown diagrammatically; these were analysed statistically by Mann-Whitney, Wilcoxon, and χ^2 tests. (2) patterns of dominance in individual *babies* (that is, a particular genus accounting for the highest count in the bacterial population examined); these were analysed by χ^2 and McNemar tests.

(1) Counts of individual organisms (figs 1 and 2)

(a) Changes with time—The faecal flora changed with time but more changes were seen in the formula fed babies. Significant changes in the number of babies colonised are shown in columns marked y:

(i) breast fed babies had an increase in coliforms other than *Escherichia coli* but a decrease in enterococci;

(ii) formula fed babies had an increase in *E coli*, enterococci, and lactobacilli but a decrease in staphylococci.

Significant changes in the counts of organisms are shown in columns marked x:

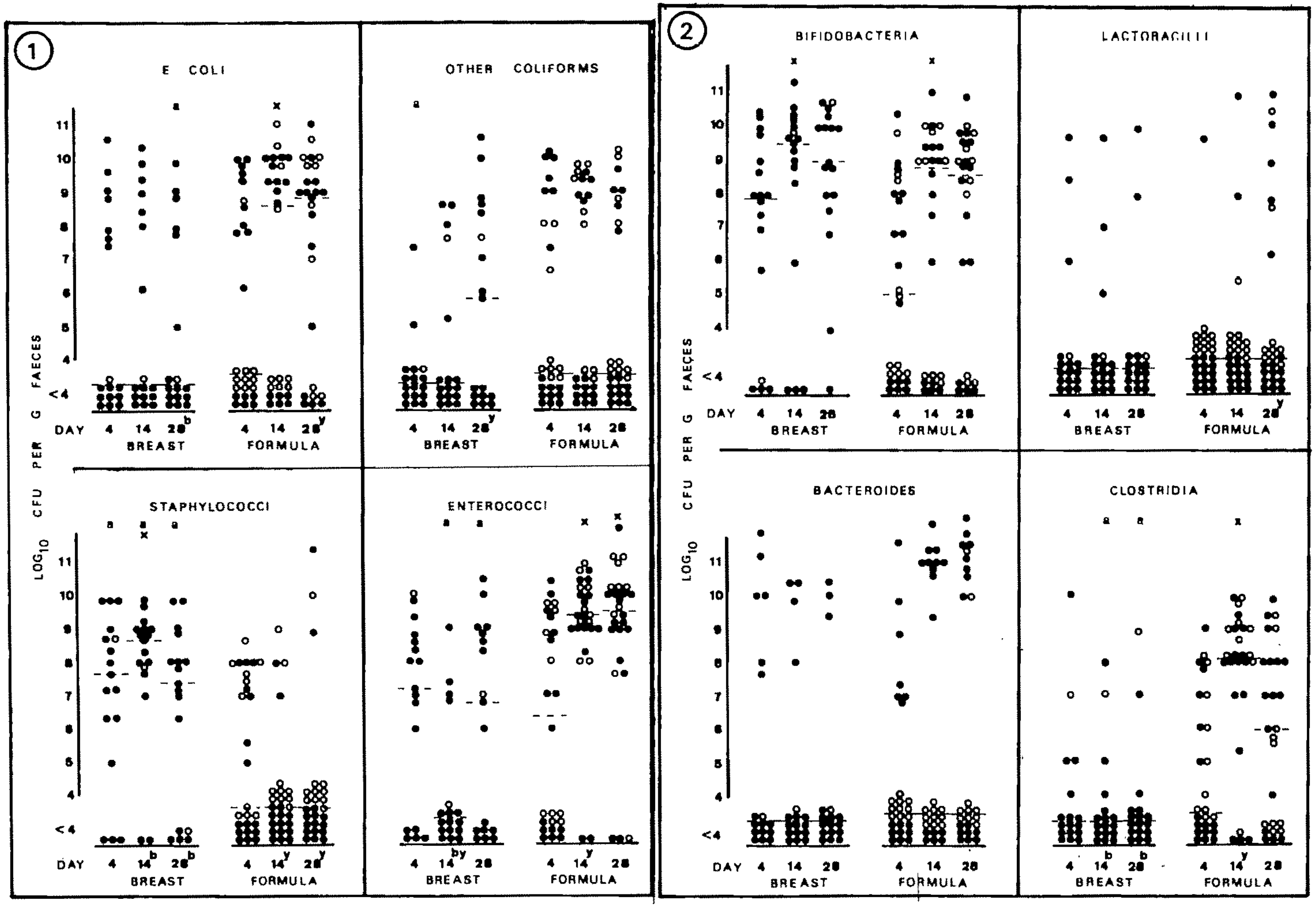
(i) breast fed babies had an increase in staphylococci and bifidobacteria;

(ii) formula fed babies had increase in *E coli*, enterococci, bifidobacteria, and clostridia.

(b) Dietary differences—More breast fed babies were colonised with staphylococci but fewer with clostridia (columns marked b).

Breast fed babies had lower counts *E coli* coliforms, enterococci, and clostridia but higher counts of staphylococci (columns marked a).

(c) Mode of delivery—Only one breast fed baby was delivered by caesarean section but 10 formula fed babies were delivered by section. None of the 10 babies were colonised by bacteroides on either day 4



Figs 1 and 2 *Counts of individual organisms* ($\log_{10}$ *colony forming units (CFU) per g of faeces) in the two dietary groups on days 4, 14, and 28. Median count shown by horizontal broken line; a, distribution of counts significantly different* ($p<0{\cdot}05$) *from counts in formula fed babies of the same age (Mann-Whitney test); b, proportion of breast fed babies in whom an organism was not detected, is significantly different* ($p<0{\cdot}05$) *from formula fed babies of the same age* (χ^2 *with Yates's correction); x, distribution of counts is significantly different* ($p<0{\cdot}05$) *from counts at day 4 (Wilcoxon); and y, proportion of babies in whom an organism was not detected is significantly different* ($p<0{\cdot}05$) *from that proportion at day 4 (McNemar with Yates's correction). ●=Babies delivered vaginally and ○=babies delivered by caesarean section.*

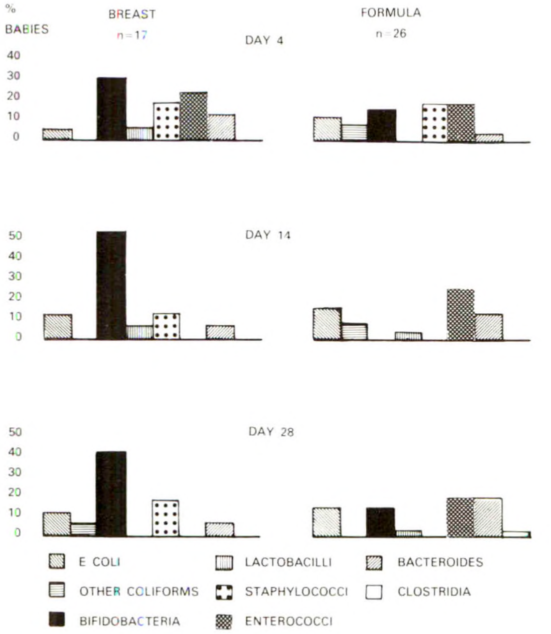


Fig 3 *Percentage of babies in whose faeces organisms were dominant.*

or day 14. Of the 16 vaginally born formula fed babies, however, seven were colonised with bacteriodes on day 4 ($p<0.02$) and 10 by day 14 ($p<0.001$).

(2) Patterns of dominance (fig 3)

(a) Changes with time—Enterococci were not dominant in any breast fed baby at days 14 or 28 and staphylococci were not dominant in any formula fed baby at days 14 and 28.

(b) Dietary differences—At day 4 no difference was observed. Later at day 14, when compared with breast fed babies, none of the formula fed babies had bifidobacteria as the dominant organism ($p<0.001$) and more had enterococci dominant, although this was not significant. This trend was continued at day 28 with an increased number of breast fed babies with bifidobacteria as the dominant organism, but it was no longer significant.

Table 3 *Mean (SD) pH of faeces*

	Breast fed	*Formula fed*
Day 4	5·56 (0·35)	5·90 (0·79)
Day 14	5·49 (0·40)*	6·91 (0·80)*†
Day 28	5·74 (1·03)*	7·07 (0·94)*†

*Difference between breast and formula fed babies $p<0.001$.
†Significant difference between value on day 4.

FAECAL PH (TABLE 3)

The pH of the breast fed babies was always more acidic than the formula fed babies and this was highly significant at days 14 and 28. The pH of the breast fed babies did not change significantly during the study but that of the formula fed babies became less acidic after day 4.

Discussion

Generally among breast fed babies bifidobacteria and staphylococci were the predominating organisms (for example, higher counts or more babies colonised or more babies in which the organisms were dominant), whereas in formula fed babies the predominant organisms were enterococci. It does seem therefore that modern infant formulas, despite extensive modification during manufacture, result in a faecal flora substantially different from that of the breast fed baby.

Table 4 summarises the results of this and some previous studies. The nature of the diets used is often not stated. However, like many other investigators we found a predominance of bifidobacteria in the breast fed babies. We are unclear why this was not observed in the babies born at St Bartholomew's Hospital, London.[3] The suggestion by the authors of that study that the antiseptic environment of modern obstetrics being responsible is an interesting one. In this hospital we use 1·5% chlorhexidine gluconate and 15% cetrimide diluted 1:50 (Savlon) for vulval washing and 1% chlorhexidine gluconate (Hibitane Obstetric cream) for vaginal examinations during labour, but antiseptic nipple sprays are not encouraged.

What are the exact dietary constituents responsible for the observed differences in the faecal flora in the formula and breast fed infant? The substances and mechanisms implicated (casein, phosphate, buffering capacity, lactoferrin, secretory IgA) and the reviews of the subject are legion,[16–18] but as breast milk differs from an infant formula in so many ways it is difficult to know which is the main factor. In the succeeding papers we have looked at the microbiological effects of specific nutrients: casein and whey proteins, phosphate, lactoferrin, and iron.

This project was partially supported by Nestlé (Nestec Ltd, Research Centre), CH 1800 Vevey, Switzerland. We are grateful to Mrs C Boyle and Mrs N Burton for the preparation of feeds and the collection of specimens.

Table 4 *Comparison of studies examining faecal microflora in breast and formula fed babies. (Results refer to breast fed babies)*

Country	*No of babies*	*Age of babies*	*Type of milk*	*Less coliforms*	*More bifidobacteria*	*Less enterococci*	*More staphylococci*	*Less bactoides and clostridia*
United Kingdom (this study)	17	4 days	Breast	No	Yes	Yes	Yes	No
	26		Nan					
	17	14 days	Breast	Yes	Yes	Yes	Yes	Yes
	26		Nan					
	17	28 days	Breast	Yes	Yes	Yes	Yes	Yes
	26		Nan					
United Kingdom[1]	7	1 week	Breast	Yes	Yes	Yes	NT	Yes
	10		Cows' milk					
	5	2 weeks	Breast	No	Yes	Yes	NT	Yes
	4		Cows' milk					
	7	3 weeks	Breast	Yes	Yes	Yes	NT	Yes
	4		Cows' milk					
	4	5 weeks	Breast	Yes	Yes	Yes	NT	Yes
	3		Cows' milk					
	2	7 weeks	Breast	No	Yes	Yes	NT	Yes
	2		Cows' milk					
United Kingdom[8]	24	7–10 days	Breast	No	Yes	NT	NT	NT
	20		SMA					
	20		Cow and Gate special					
	10		Cow and Gate ½ cream					
	4		Golden Ostermilk					
United Kingdom[9]	11	5–7 days	Breast	No	Yes	NT	NT	NT
	21		SMA Gold Cap					
United Kingdom[10]	9	1–5 days	Breast	No	Yes	NT	NT	No
	6		Formula*					
	9	3 weeks	Breast	No	No	NT	NT	No
	6		Formula*					
	9	6 weeks	Breast	No	No	NT	NT	No
	6		Formula*					
United Kingdom[3]	15	2 weeks	Breast	No	No	Yes	Yes	Yes
	11		Formula*					
	16	4 weeks	Breast	No	No	Yes	Yes	Yes
	12		Formula*					
	15	6 weeks	Breast	No	No	Yes	Yes	Yes
	12		Formula*					
Nigeria	12		Breast	Yes	Yes	No	No	Yes
Sweden[4]	15	5 days	Breast	No	Yes	Yes	Yes	Yes
	7		Whey based formula					
	15	3 weeks	Breast	Yes	No	Yes	Yes	No
	7		Whey based formula					
	15	8 weeks	Breast	No	No	No	Yes	No
	7		Whey based formula					
Australia[11]	7	1 week	Breast	Yes	Yes	Yes	NT	Yes
	7	4 weeks	Formula*					
Japan[12]	70	28–46 days	Breast	Yes	No	Yes	No	Yes
	35		Formula*					
Japan[13]	6	1–6 days	Breast	Yes	Yes	Yes	No	Yes
	7		Cows' milk					
	6	4 weeks	Breast	No	Yes	Yes	No	Yes
	7		Cows' milk					
France[14]	22	1–8 days	Breast	No	Yes	No	NT	No
	11		Gallia					
Holland[15]	10	1–3 months	Breast	Yes	Yes	Yes	NT	No
	6		Almiron					
	7		Almiron—no added iron	Yes	No	Yes	NT	No

*Formula not specified; NT=not tested.

References

1 Bullen CL, Tearle PV, Willis AT. Bifidobacteria in the intestinal tract of infants—an in vivo study. *J Med Microbiol* 1976;**9**:325–33.

2 Wharton BA. Infant formulae. *Bulletin British Nutrition Foundation* 1984;9:83–93.

3 Simhon A, Douglas JR, Drasar BS, Soothill JF. Effect of feeding on infants faecal flora. *Arch Dis Child* 1982;**57**:54–8.

4 Lundequist B, Nord CE, Winberg J. The composition of faecal microflora in breast fed and bottle fed infants from birth to 8 weeks. *Acta Paediatr Scand* 1985;**74**:45–51.

5 Cowan ST. *Manual for the identification of medical bacteria*. Cambridge: Cambridge University Press, 1981.

6 Holdeman LV, Cato EP, Moore WEC. *Anaerobe laboratory manual*. Virginia: Virginia Polytechnic Institute, 1977.

7 Mitsuoka T, Kaneuchi C. Ecology of the bifidobacteria. *Am J Clin Nutr* 1977;**30**:1799–810.

8 Hewitt JH, Rigby J. Effects of various milk feeds on numbers of E. coli and Bifidobacterium in the stools of newborn infants. *J Hyg (Camb)* 1976;**77**:129–39.

9 Dolby JM, Honour P, Valman HB. Bacteriostasis of E coli by milk. I. Colonisation of breast fed infants by milk resistant organisms. *J Hyg (Camb)* 1977;**78**:85–94.

10 Rose SJ. Bacterial flora of breast fed infants. *Pediatrics* 1984;**74**:563–4.

11 Stark PL, Lee A. The microbial ecology of the large bowel of breast fed and formula fed infants during the first year of life. *J Med Microbiol* 1982;**15**:189–203.

12 Benno Y, Sawada K, Mitsuoka T. The intestinal microflora of infants: composition of fecal flora in breast fed and bottle fed infants. *Microbiol Immunol* 1984;**28**:975–86.

13 Yoshioka H, Iseki K, Fujita J. Development and differences of intestinal flora in the neonatal period in breast fed and bottle fed infants. *Pediatrics* 1983;**72**:317–21.

14 Moreau MC, Thomasson M, Ducluzeau R, Raibaud P. Cinetique d'establissement de la microflora digestive chez le nouveau—ne humain en fonction de la nature du lait. *Reprod Nutr Dev* 1986;**26(2B)**:745–53.

15 Mevissen-Verhage EAE, Marcelis JH, Harmsen-Van Amerongen WCM, de Vos NM, Verhoef J. Effect of iron on neonatal gut flora during the first three months of life. *Eur J Clin Microbiol* 1985;**4**:273–8.

16 Bullen JJ. Role of milk and gut flora in the protection of the newborn against infection. In: Lambert HP, Wood CBS, eds. *Immunological aspects of infection in the fetus and the newborn*. London: Academic Press, 1981:123–37.

17 Rolles CJ, Hall MA, Duncan H, Sampeys C. Bifidobacteria in breast milk—a review and a hypothesis. In: Wharton BA, ed. *Topics in perinatal medicine*. Vol 2. Bath: Pitman, 1982:31–47.

18 Wharton BA. Immunological implications to alternatives to mother's milk. 1. Infant formulas. In: Wilkinson AR, ed. *Immunology of infant feeding*. New York: Plenum Press, 1981:107–22.

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Diet and faecal flora in the newborn: casein and whey proteins

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SUMMARY Despite the extensive modifications of cows' milk to make an infant formula resemble human breast milk, we showed in a previous study that the faecal flora of breast fed babies still differs substantially from that of formula fed babies. This paper describes the effects that differences in the distribution of whey proteins and caseins exert on the faecal flora. Faecal flora was examined in 33 babies receiving a whey formula, 29 babies receiving a casein formula, and 38 breast fed babies. Subsequently fewer babies in each group were studied at weeks 7, 11, and 15. More whey fed babies were colonised with bifidobacteria at 14 days compared with the casein fed group, more casein fed babies were colonised with bacteroides (at 14 days), and more had a dominant growth of enterococci (at week 7). It seems therefore that during the first two months that the whey predominant formula induced a faecal flora generally closer to that of breast fed babies than did a casein formula.

In the preceeding paper it was shown that among breast fed babies bifidobacteria and staphylococci were the predominating organisms in the faeces whereas in bottle fed babies the predominating organisms were enterococci and *Escherichia coli.*[1]

Breast milk differs from a modern infant formula in many ways so that the dietary cause(s) for these microbiological differences is (are) not clear. This present investigation was therefore designed to determine the effects of protein quality (in particular the distribution of whey proteins and caseins) on the faecal flora. Few hospitals use a casein predominant formula but many mothers change their baby to a casein predominant one after some weeks and often following professional advice. The study therefore also provided an opportunity to determine the faecal flora of the substantial minority of babies who receive casein predominant formulas in the early weeks of life.

Table 1 *Composition of the two test formulas and breast milk (per l)*

	Breast milk	*Whey formula (Gold Cap)*	*Casein formula (White Cap)*
Protein g (nitrogen × 6·38)	11	15	15
Total 'true' protein (%)	9 (100)	13 (100)	14 (100)
Caseins	2·5 (28)	5·2 (40)	11·1 (79)
Whey proteins	6·5 (72)	7·8 (60)	2·9 (21)
α Lactalbumin	2·6 (29)	1·2 (9)	0·6 (4)
β Lactoglobulin	—	3·4 (26)	1·3 (9)
Other whey	3·9 (43)	3·2 (25)	1·0 (8)
Calcium (mg)	350	450	560
Phosphorus (mg)	150	330	450
Sodium (mg)	150	150	200
Potassium (mg)	600	560	740
Iron (mg)	0·76	6·7	6·7

Proteins for breast milk based on Hambraeus[5] and for formulas they were calculated from manufacturers information and Jennes.[6] Minerals for breast milk based on DHSS, 1977.[7]

Subjects and methods

DIET AND BABIES (TABLES 1 AND 2)

On one postnatal ward between January and December 1987, babies, whose mothers had opted to bottle feed them, were allocated to receive either a whey predominant formula or a casein predominant one. The whey predominant formula used was Gold Cap SMA—known as S26 in some countries (Wyeth). The casein predominant formula was White Cap SMA (Wyeth). The test formula offered on the postnatal ward was alternated at approximately monthly intervals—for example, all babies born during July were offered the whey formula, all those born during August were offered the casein formula, etc. Once allocated to a formula the babies

Table 2 *Data of babies taking part in the study*

	Breast fed	*Formula fed*	
		Whey	*Casein*
Day 14:			
No of babies	38	33	29
Sex:			
Male	20	15	12
Female	18	18	17
Race:			
White	35	32	26
Asian	0	0	1
Afro-Carribean	3	1	2
Mean (SD) birth weight (g)	3360 (410)	3520 (490)	3340 (380)
Week 7:			
No of babies	15	17	16
Week 11:			
No of babies	10	10	13
Week 15:			
No of babies	10	9	13

remained on that formula (which was supplied free of charge) until they left the study. The formulas were supplied as 'ready to feed' liquid milk until the baby was 3 weeks old and thereafter the mothers were supplied with dried milk powder and they reconstituted the formula themselves. Breast fed babies were also studied and at the end of the study their mothers were given a voucher to purchase goods from a pharmacist's shop.

Compared with the casein formula, the whey formula contains more β lactoglobulin, less casein, and (as casein is associated with both phosphorus and calcium) less phosphorus and calcium. The demineralisation process also results in lower electrolyte concentrations similar to those in breast milk. Both formulas contained added iron (table 1).

All babies were born at term (none less than 37 weeks' gestation) and all except one were born vaginally. The babies in the three groups were similar in size, etc (table 2).

PROCEDURES

Faeces were collected on day 14 and at week 7, 11, and 15. Anthropometry was recorded on day 1. The methods used for microbiology and pH of faeces were exactly as described previously.[1]

STATISTICAL ANALYSIS

The results were analysed statistically using Mann-Whitney, Wilcoxon, χ^2, and Student's *t* test as appropriate.

ETHICAL APPROVAL

Approval for the study was obtained from the ethics committee of the South Birmingham Health Authority. The mothers of all babies taking part in the study gave written informed consent.

IN VITRO STUDY

The milk pH was measured using a standard pH meter (Radiometer PHM82) and the pH converted also to hydrogen ion concentration (nmol/l). Titration methods for the measurement of titratable acidity and buffering capacity were based on those of Harrison and Peat[2] and Bullen and Willis.[3] Titratable acidity was defined as the volume (ml) of 0·5 mol/l sodium bicarbonate required to raise the pH of the milk from its initial value to 7·40, expressed per litre of milk. Buffering capacity was defined as the volume (ml) of 0·1 mol/l hydrochloric acid required to reduce the milk pH from its initial value to 5·0, expressed per litre of milk. Bicarbonate was measured by the method of Ibbott *et al*,[4] and osmolality using a Roebling Automatik Osmometer, each expressed as mmol/l. Citrate was measured using a citric acid test kit produced by BCL London Ltd, expressed as mmol/l.

Results

For ease of reference the breast fed babies are often referred to as 'breast babies' or 'breast group' and similarly for the formula fed babies. All except one baby was delivered vaginally (see methods) so this variable does not have to be considered.

MICROBIOLOGY

Differences were seen mainly at day 14 and these results are presented in detail but results for follow up studies are also described. In the same way as in the preceding paper the results are shown in two ways: (1) counts of individual *organisms* examined in each baby, analysed statistically using the Mann-Whitney test and the presence or absence of an organism, analysed by the χ^2 test (fig 1, tables 3 and 4). (2) Patterns of dominance in individual *babies* (that is, a particular genus accounting for the highest count in the bacterial population examined); these were analaysed statistically by the χ^2 test (fig 2).

Day 14

(1) Counts of individual organisms (fig 1)

Differences in the three dietary groups were seen only in the counts of staphylococci and bifidobacteria; no significant differences were seen with the other organisms.

Significant differences in the counts of organisms are shown in columns marked a and b:

(i) both groups of formula fed babies had lower

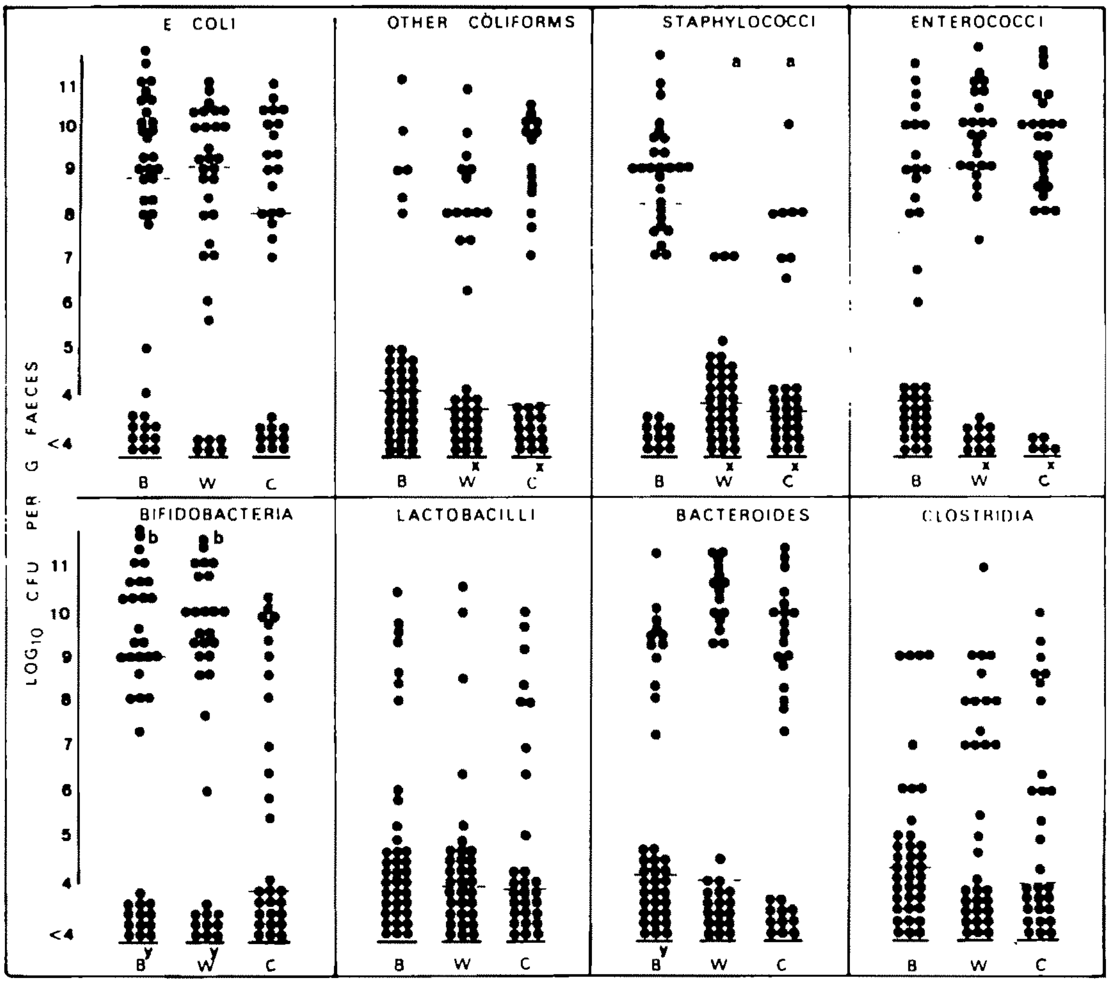

Fig 1 *Counts of individual organisms ($\log_{10}$ colony forming units (CFU) per g faeces) in the three dietary groups on day 14. a, Distribution of counts significantly different ($p<0{\cdot}05$) form breast fed babies (Mann-Whitney); b, distribution of counts significantly different ($p<0{\cdot}05$) from casein babies (Mann-Whitney); x, proportion of babies colonised significantly different ($p<0{\cdot}05$) from breast fed babies (χ^2 with Yates's correction); and y, proportion of babies colonised significantly different ($p<0{\cdot}05$) from casein babies (χ^2 with Yates's correction). B=breast fed babies, W=whey fed babies, and C=casein fed babies.*

counts of staphylococci than the breast fed babies ($p<0{\cdot}05$);

(ii) the breast fed and whey fed babies had higher counts of bifidobacteria than the casein group ($p<0{\cdot}05$).

More dietary differences were seen when the number of babies colonised, or not, were compared. Significant changes are shown in columns marked x and y:

(i) fewer breast fed babies were colonised with other coliforms and enterococci than both of the formula fed groups ($p<0{\cdot}05$);

(ii) more breast fed babies were colonised with staphylococci than both of the formula fed groups ($p<0{\cdot}05$);

(iii) fewer casein babies were colonised with bifidobacteria compared with both breast and whey fed groups ($p<0{\cdot}05$);

Table 3 *Comparison of faecal flora at week 7*

	Breast fed (n=15)	*Formula fed*	
		Whey (n=17)	*Casein (n=16)*
Escherichia coli:			
No (%) of babies colonised	9 (60)	15 (88)	12 (75)
Median count	0·0001	0·5	0·04
Range of counts (× 10^9/g)	0–30	0–20	0–600
Other coliforms:			
No (%) of babies colonised	7 (47)	2 (13)	6 (38)
Median count	0	0	0
Range of counts (× 10^9/g)	0–100	0–8	0–0·7
Bifidobacteria:			
No (%) of babies colonised	10 (67)	13 (76)	10 (63)
Median count	0·8	2·0	0·1
Range of counts (× 10^9/g)	0–10	0–90	0–20
Lactobacilli:			
No (%) of babies colonised	3 (20)	3 (18)	4 (25)
Median count	0	0	0
Range of counts (× 10^9/g)	0–10	0–0·1	0–10
Staphylococci:			
No (%) of babies colonised	6 (40)*	0*‡	5 (31)‡
Median count	0	0	0
Range of counts (× 10^9/g)	0–0·1	0	0–3
Enterococci:			
No (%) of babies colonised	8 (53)*†	16 (94)*	15 (94)†
Median count	0·006	1·0	0·7
Range of counts (× 10^9/g)	0–40	0–1	0–1700
Bacteroides:			
No (%) of babies colonised	5 (33)	12 (71)	7 (44)
Median count	0	4·0	0
Range of counts (× 10^9/g)	0–40	0–100	0–30
Clostridia:			
No (%) of babies colonised	8 (53)	9 (53)	14 (88)
Median count	0	0·00001	0·01
Range of counts (× 10^9/g)	0–1	0–1	0–0·3

Significant differences by χ^2 test: *differences between babies fed breast milk and whey formula; †differences between babies fed breast milk and casein formula; and ‡differences between babies fed casein and whey formulas.

(iv) more casein babies were colonised with bacteroides compared with breast fed babies ($p<0{\cdot}05$).

(2) Patterns of dominance (fig 2)

Dominance of bifidobacteria occurred in more breast fed than formula fed babies ($p<0{\cdot}05$). Although not significant more whey babies (15%) had bifidobacteria as the dominant organisms compared with the casein group (7%).

Follow up study—weeks 7, 11, and 15 (tables 3 and 4)

There were very few significant changes and dietary differences in the faecal flora examined in subsequent specimens collected during the follow up study.

(1) Counts of individual organisms

There was no difference in the counts of individual organisms between the breast fed and either of the formula fed groups. There were a few dietary differences in the presence or absence of organisms:

(i) more breast fed babies (40%) and casein babies (31%) were colonised with staphylococci at week 7 than whey babies (0%) ($p<0{\cdot}05$, $p<0{\cdot}05$);

(ii) fewer breast fed babies (53%) were colonised with enterococci at week 7 than either of the formula fed groups (94%) ($p<0{\cdot}05$);

(iii) more breast fed babies (60%) and whey fed babies (56%) were colonised with other coliforms at 15 weeks than casein babies (11%) ($p<0{\cdot}04$, $p<0{\cdot}06$

(2) Patterns of dominance (fig 2)

Significant dietary differences in the dominance patterns were seen only at week 7. Apparent differences seen at week 15 (see fig 2) were not significant. More casein babies (44%) had a dominance of enterococci than breast babies (7%) at week 7 ($p<0{\cdot}05$).

Table 4 *Comparison of faecal flora at weeks 11 and 15*

	Breast fed (*n=10*)	*Formula fed*	
Week 11		*Whey* (*n=10*)	*Casein* (*n=13*)
Escherichia coli:			
No (%) of babies colonised	7 (70)	9 (90)	11 (85)
Median count	0·05	1·05	2
Range of counts (× 10^9/g)	0–10	0–30	0–20
Other coliforms:			
No (%) of babies colonised	6 (60)	4 (40)	6 (46)
Median count	0·25	0	0
Range of counts (× 10^9/g)	0–7	0–1	0–10
Bifidobacteria:			
No (%) of babies colonised	7 (70)	9 (90)	9 (69)
Median count	0·5	1·0	1·0
Range of counts (× 10^9/g)	0–50	0–30	0–20
Lactobacilli:			
No (%) of babies colonised	2 (20)	2 (20)	2 (15)
Median count	0	0	0
Range of counts (× 10^9/g)	0–0·2	0–0·04	0–0·3
Staphylococci:			
No (%) of babies colonised	3 (30)	2 (20)	2 (15)
Median count	0	0	0
Range of counts (× 10^9/g)	0–0·8	0–5	0–1
Enterococci:			
No (%) of babies colonised	9 (90)	9 (90)	11 (85)
Median count	0·65	0·15	1·0
Range of counts (× 10^9/g)	0–6	0–20	0–20
Bacteroides:			
No (%) of babies colonised	3 (30)	7 (70)	5 (38)
Median count	0	0·22	0
Range of counts (× 10^9/g)	0–9	0–20	0–30
Clostridia:			
No (%) of babies colonised	6 (60)	7 (70)	8 (62)
Median count	0·000005	0·00055	0·01
Range of counts (× 10^9/g)	0–9	0–1	0–0·1
Week 15	(*n=10*)	(*n=9*)	(*n=13*)
Escherichia coli:			
No (%) of babies colonised	9 (90)	4 (44)	13 (100)
Median count	0·51	0	1
Range of counts (× 10^9/g)	0–6	0–40	0–30
Other coliforms:			
No (%) of babies colonised	6 (60)	5 (56)*	2 (11)*
Median count	0·01	0·0001	0
Range of counts (× 10^9/g)	0–10	0–10	0–0·1
Bifidobacteria:			
No (%) of babies colonised	8 (80)	5 (56)	8 (62)
Median count	0·05	0·004	0·9
Range of counts (× 10^9/g)	0–700	0–10	0–20
Lactobacilli:			
No (%) of babies colonised	2 (20)	1 (11)	3 (23)
Median count	0	0	0
Range of counts (× 10^9/g)	0–1	0–0·04	0–1
Staphylococci:			
No (%) of babies colonised	4 (40)	2 (22)	3 (23)
Median count	0	0	0
Range of counts (× 10^9/g)	0–1	0–0·02	0–0·2
Enterococci:			
No (%) of babies colonised	10 (100)	8 (89)	13 (100)
Median count	1·5	1	2
Range of counts (× 10^9/g)	0–30	0–10	0–10
Bacteroides:			
No (%) of babies colonised	4 (40)	3 (33)	9 (69)
Median count	0	0	0·2
Range of counts (× 10^9/g)	0–80	0–20	0–20
Clostridia:			
No (%) of babies colonised	10 (100)	5 (55)	10 (77)
Median count	0·055	0·00001	0·01
Range of counts (× 10^9/g)	0–10	0–20	0–0·5

Significant differences by χ^2 test*: *differences between babies fed casein and whey formulas.

Table 5 *Mean (SD) pH of faeces*

	Breast fed	*Formula fed*	
		Whey	*Casein*
Day 14:			
No of babies	38	33	29
pH	6·18 (0·67)*†	6·87 (0·71)*	7·12 (0·58)†
Week 7:			
No of babies	15	17	16
pH	6·92 (1·15)	7·11 (0·78)	7·44 (0·54)
Week 11:			
No of babies	10	10	13
pH	6·99 (0·90)	7·23 (0·70)	7·63 (0·73)
Week 15:			
No of babies	10	9	13
pH	6·89 (1·08)	7·22 (0·84)	7·26 (0·69)

Significant differences using *t* test: *difference between babies fed breast milk and whey formula ($p<0·01$) and †difference between babies fed breast milk and casein formula ($p<0·001$).

Changes with time

Changes in time were studied in each baby by analysing the results with Wilcoxon test but no significant changes in the faecal microflora were found on any of the diets throughout the 15 weeks of the study.

FAECAL PH (TABLE 5)

The breast babies had consistently more acidic faeces than the formula babies, although this was only significant on day 14. There was a tendency for the breast fed babies faeces to become less acidic throughout the period of the study but the change from day 14 was only significant at week 11 ($p=0·04$). There was no change of the pH of the faeces of the formula fed babies and there was no difference in the pH between the whey and the casein fed babies.

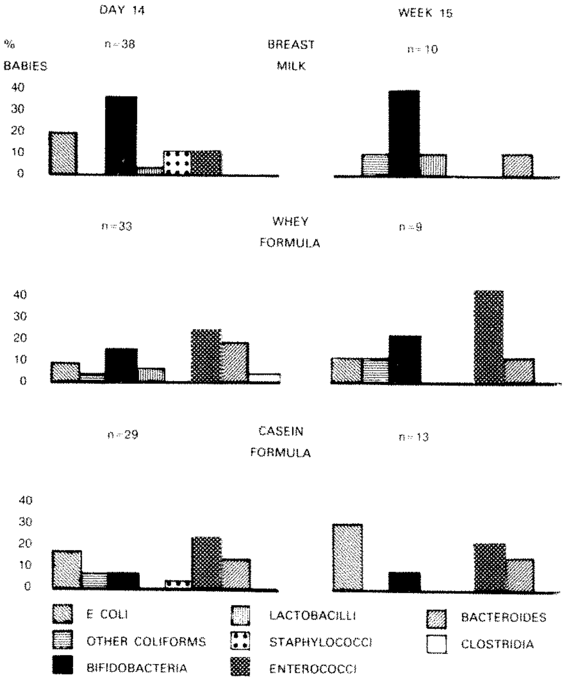

Fig 2 *Percentage of babies in whose faeces organisms were dominant at day 14 and week 15.*

IN VITRO STUDY (TABLE 6)

The casein formulas compared with the whey ones had higher values for hydrogen ion and titratable acidity (and to this extent were closer to breast milk) and had higher values for buffering capacity and citrate (and to this extent were further away from breast milk). The differences were more noticeable in the powder formulas than the liquid ones with the exception of bicarbonate.

Table 6 *Acid base properties of the milks used in the study*

	Cows' milk	*Breast milk**	*Whey formula*		*Casein formula*	
			Liquid	*Powder*	*Liquid*	*Powder*
Milk pH	6·68	6·59	6·73	7·05	6·69	6·75
Hydrogen ion concentration (nmol/l)	210	257	186	89	204	178
Titratable acidity†	208	122	110	53	147	133
Buffering capacity‡	36	12	15	17	16	20
Bicarbonate (nmol/l)	5·4	0·5	2·5	2·0	1·0	2·3
Citrate (nmol/l)	12·4	2·8	2·9	4·3	4·5	5·1
Osmolality (nmol/l)	282	290	307	332	307	326

*Breast milk sample from mother who had been lactating for six weeks.
†Volume (ml) of 0·5 mol/l sodium bicarbonate required to raise the pH of the milk from its initial value to 7·40 (expressed per litre of milk).
‡Volume (ml) of 0·1 mol/l hydrochloric acid required to reduce the milk pH from its initial value to 5·0 (expressed per litre of milk).

Discussion

At 2 weeks of age more babies receiving the whey formula were colonised with bifidobacteria and had higher counts of bifidobacteria than those fed the casein formula. In addition when compared with the breast group more casein babies were colonised with bacteroides (at day 14) and more had a dominant growth of enterococci (at week 7). These differences were not seen in the whey groups. It seems that a whey formula induces a faecal flora closer to that of breast fed babies than does a casein formula. The only observation against this general conclusion was that at week 7 more casein and breast babies were colonised with staphylococci than whey babies.

These dietary effects are presumably due to the differences in the casein and whey content of the two formulas. The casein content is also associated, however, with other differences, in particular the amount of phosphorus and calcium. Casein forms a micellar association with phosphorus and calcium, so generally any milk with a high casein content will have a higher phosphate content too. Both casein and phosphate are said to increase the buffering capacity of the food so that the intestinal contents are less acidic favouring the growth of organisms other than bifidobacteria.[3] Certainly the phosphorus concentration was shown to have an effect upon microbial activity in an in vitro study using a continuous culture technique with the rumen contents of sheep.[8]

The results of our in vitro study indicated that the pH and the titrable acidity of the formula bore little relationship to that of the faeces. We found it difficult to relate the observed in vivo microbiological differences in the babies to the in vitro acid base differences in the formulas (table 6). We cannot confirm therefore that the differences in the microbiological effects of whey and casein are mediated via an acid base mechanism.

Casein apart, the whey proteins of breast milk are very different from the whey proteins of cows' milk. In particular the whey formula contained much more β lactoglobulin and the whey proteins contain little lactoferrin. In vitro experiments suggest that lactoferrin has a specific effect on gut flora and this is examined in more detail in the next paper.

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References

[1] Balmer SE, Wharton BA. Diet and faecal flora in the newborn: breast milk and infant formula. *Arch Dis Child* 1989;**64**:1672–7.

[2] Harrison C, Peat G. Significance of milk pH in newborn infants. *Br Med J* 1972;**iv**:515–8.

[3] Bullen CL, Willis AT. Resistance of the breast fed infant to gastroenteritis. *Br Med J* 1971;**iii**:338–43.

[4] Ibbott AF, Laganga TS, Gin JB, Inkpen JA. Blood, pH, CO_2 and O_2. In: Henry RJ, Cannon DC, Winkelman JW, eds. *Clinical chemistry: principles and technics*. London: Harper and Row, 1974:786–8.

[5] Hambraeus L. Human milk composition. *Nutrition Abstracts Review A* 1984;**54**:219–36.

[6] Jennes R, Sloan RE. The composition of milks of various species: a review. *Dairy Science Abstracts* 1970;**32**:599–612.

[7] Department of Health and Social Security. *Composition of mature human milk*. (Report 12.) London: HMSO, 1977.

[8] Komisarczuk S, Merry RJ, McAllan AB. Effects of phosphorus on rumen microbial fermentation and synthesis determined using a continuous culture technique. *Br J Nutr* 1987;**57**:279–90.

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Diet and faecal flora in the newborn: lactoferrin

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SUMMARY The faecal flora of breast fed babies differs from that of bottle fed babies. We have shown that the use of a whey predominant formula rather than a casein predominant one induced a faecal flora generally closer to that of breast fed babies but substantial differences remained. The whey proteins of breast milk include much more lactoferrin than is found in cows' milk. Observations both in animals and in vitro suggest that lactoferrin could be responsible for some of these differences between bottle and breast fed babies. This study was designed to determine the effects on faecal flora of the addition of bovine lactoferrin to the diet of bottle fed babies while holding other qualities of their diet constant. As lactoferrin is an iron binding protein three test formulas were used: (a) no added iron and no added lactoferrin (basic), (b) no iron but added lactoferrin (L), and (c) added iron and lactoferrin (LF). The addition of lactoferrin had little effect upon the faecal microflora and did not move the pattern of the faecal flora in the direction of the breast fed baby. The addition of iron to the formula had more effect on the faecal flora than did lactoferrin. At day 4 it encouraged *Escherichia coli* and discouraged staphylococcal faecal colonisation. At day 14 the addition of iron to the formula discouraged bifidobacteria. The reasons why bovine lactoferrin was ineffective in vivo in this study are discussed.

We have shown previously that the faecal microflora of breast fed babies differs from that of formula fed babies and contains, in particular, more bifidobacteria and fewer *Escherichia coli.*[1 2] One dietary factor responsible for this microbiological difference is the distribution of whey proteins and casein. Breast milk contains more whey proteins than casein. We have shown in the very young infant that compared with a casein predominant formula, a whey predominant infant formula results in a faecal flora closer to that of the breast fed baby. Nevertheless the faecal flora of the baby receiving a whey predominant formula is still substantially different from that of a breast fed baby. The whey proteins of cows' milk differ considerably from those of human milk. One difference is the presence in human milk of lactoferrin in relatively high concentrations (100–600 mg/100 ml). Lactoferrin has a high affinity for the small amounts of iron in breast milk so that the iron is unavailable for the multiplication of organisms. Lactobacilli are not affected by this mechanism, however, as they are one of the few organisms that thrive without iron.[3] The bacteriostatic effect of lactoferrin is negated if extra iron is added to the system as this saturates the lactoferrin and leaves free iron available for the growth of other organisms.[4] This explanation is, however, based mostly on in vitro observations, although a few have been made in animals.[5 6] Bovine lactoferrin is now available in amounts sufficient for use in experimental situations. This has given us the opportunity to study in human babies the effects on faecal flora of the addition of lactoferrin.

Subjects and methods

DIET AND BABIES (TABLES 1 AND 2)

Table 1 gives details of the three formulas that were manufactured in special batches for the study by Nestlé. All formulas were based on demineralised whey, skimmed milk, and an identical fat blend. The 'basic' formula contained neither added lactoferrin nor added iron. The L formula consisted of basic plus lactoferrin and the LF formula consisted of basic plus lactoferrin plus iron.

Mothers on the postnatal wards of Sorrento Maternity Hospital, Birmingham, between January 1985 and March 1986, who had decided not to breast feed their babies, were approached by the project nurses. If they gave informed written consent their babies were allocated on a rotational basis to one of the three test formulas and remained on that

Table 1 *Composition of three test formulas and breast milk (per l)*

	Breast milk	*Formula*		
		Basic	*L*	*LF*
Protein g (nitrogen×6·38)	11	17	19·8	19·8
Total 'true' protein (%)	9 (100)	16 (100)	19 (100)	19 (100)
Caseins	2·5 (28)	6·4 (40)	6·4 (34)	6·4 (34)
Whey proteins	6·5 (72)	9·6 (60)	9·6 (66)	9·6 (66)
α Lactalbumin	2·6 (29)	1·6 (9)	1·6 (8)	1·6 (8)
β Lactoglobulin	0	4·2 (26)	4·2 (22)	4·2 (22)
Serum albumin	0·5 (6)	0·4 (3)	0·4 (2)	0·4 (2)
Immunoglobulins	1·0–IgA (11)	1·4 (9)	1·4 (7)	1·4 (7)
Lysozyme	0·5 (6)	0	0	0
Human lactoferrin	1·8 (20)	0	0	0
Added bovine lactoferrin (g)	0	0	2·8 (15)	2·8 (15)
Iron (mg)	0·76	0·4	0·8	9·16
Phosphorus (mg)	150	300	300	300

Table 2 *Data of babies taking part in the study*

	Formula		
	Basic (n=20)	*L (n=18)*	*LF (n=20)*
Sex:			
Male	11	6	12
Female	9	12	8
Race:			
White	13	14	12
Asian	4	4	[illegible]
Afro-Caribbean	3	0	[illegible]
Mode of delivery:			
Normal vaginal	15	12	12
Caesarean section	5	6	8
Mean (SD) birth weight (g)	3230 (530)	3050 (480)	3[illegible]20 (470)
Haematology on day 14:			
Mean (SD) haemoglobin (Hb) (g/l)	172·1 (25·9)	183·7 (24·0)	171·9 (18·0)
Mean (SD) erythrocyte protoporphyrin (μg/g Hb)	1·67 (1·04)	1·45 (0·67)	1·42 (0·57)

formula for 14 days. The powdered formulas were reconstituted by one of two project nurses and delivered to the babies' mothers in hospital and at home. Feeds were given on demand. Babies were fed one of the special formulas for 14 days.

PROCEDURES

Anthropometric measurements were made on day 1. Faeces were collected on days 4, 11, and 14. The specimens collected on days 4 and 14 were used to measure the bacterial flora and the 24 hour specimen collected on day 11 was used to estimate the faecal lactoferrin concentration. A heelprick specimen of blood was taken on day 14.

MICROBIOLOGICAL METHODS

The methods used were exactly as described previously.[1]

FAECAL BOVINE LACTOFERRIN

The faeces were homogenised in 100 ml phosphate buffered saline, centrifuged and filtered through Whatman glass microfibre filters GF/D. The filtrate diluted 1 in 200 was used in a two site solid phase enzyme immunoassay using labelled polystyrene beads.

Beads, washed in absolute alcohol and rinsed three times in phosphate buffered saline, were labelled with antibovine lactoferrin antibody (raised in a rabbit by Dr SJ Fairweather-Tait, Food Research Institute, Norwich) by incubating at 5°C overnight, and 200 μl myoglobin (20 g/l) was added and incubated at 37°C for 1·5 hours. Beads were ready for use in the assay after three washes with phosphate buffered saline.

The assay was performed in glass test tubes with one glass bead to which 150 μl 5% albumin and 20 μl filtrate or standards were added. Bovine lactoferrin standards were supplied by Nestlé. Incubation was carried out overnight on an orbital shaker at room temperature. After washing, 300 μl lactoferrin conjugate (Serotec) was added and incubated at 37°C for 1·5 hours. After further washing the bead was transferred to a polystyrene test tube, 300 μl o-phenylenediamine was added, and it was incubated in the dark for 30 minutes. The reaction was stopped with the addition of 1·5 ml M hydrochloric acid. Absorbance was measured at 410 nm. The results are expressed as mg lactoferrin per day.

STATISTICAL ANALYSIS

The results were analysed statistically using Student's *t*, Mann-Whitney, χ^2, and Fisher's exact test as appropriate.

ETHICAL APPROVAL

Approval for the study was obtained from the ethics committee of the South Birmingham Health Authority.

Results

MICROBIOLOGY

Results are presented in detail in the same way as in the two preceding papers: (1) the counts of individual *organisms* examined in each baby, analysed statistically using the Mann-Whitney test and the presence or absence of an organism analysed by the

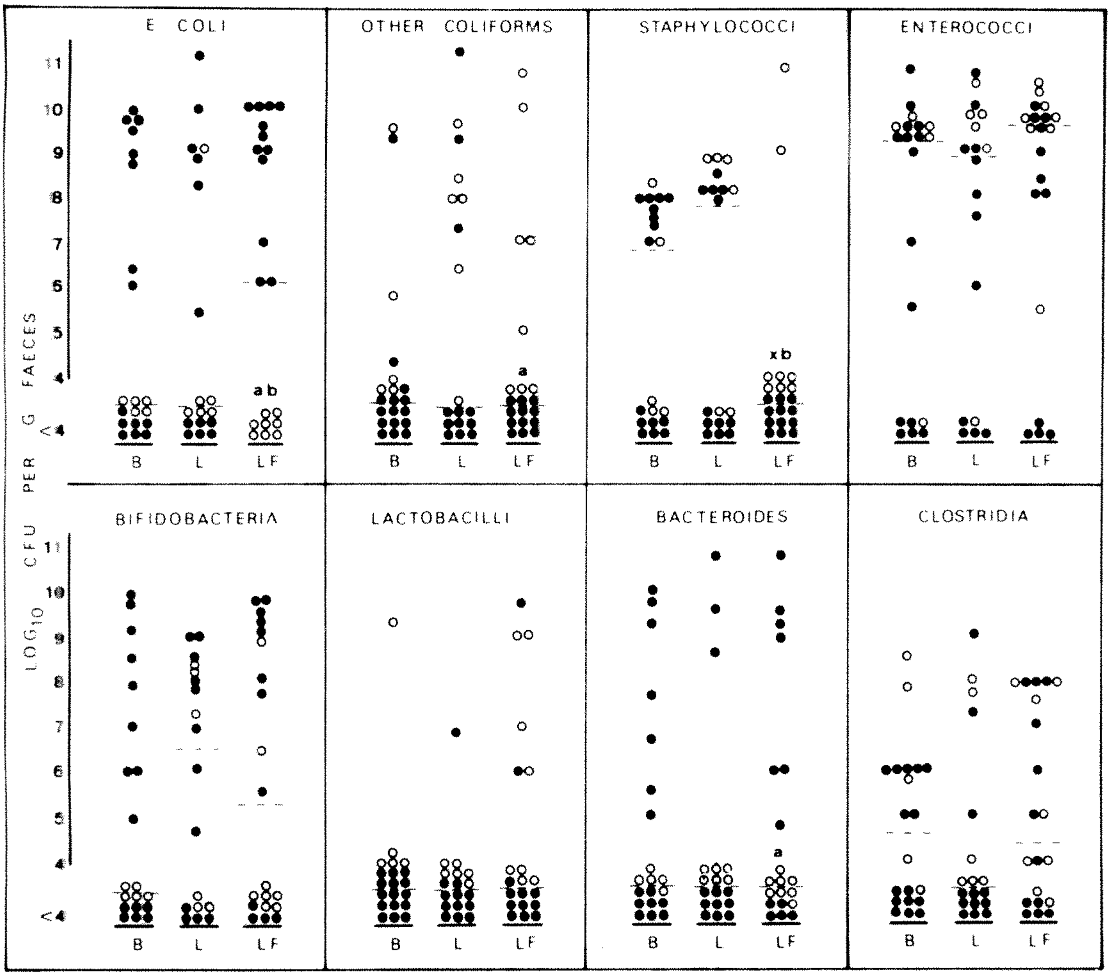

Fig 1 *Counts of individual organisms* (log_{10} *colony forming units (CFU) per g of faeces) in the three dietary groups (basic (B), L, and LF formulas) on day 4. Median count shown by horizontal broken line. a, Proportion of section delivered babies in whom an organism was not detected is significantly different* ($p<0·05$) *from vaginally delivered babies in the same dietary group* (χ^2 *with Yates's correction); b, proportion of vaginally delivered babies in whom an organism was not detected is significantly different* ($p<0·01$) *for vaginally delivered babies fed either basic or L formula* (χ^2 *with Yates's correction); x, proportion of babies, regardless of delivery, in whom staphylococci were not detected is significantly greater* ($p<0·05$) *than those fed either basic or L formula* (χ^2 *with Yates's correction).* ● = *Babies delivered vaginally and* ○ = *babies delivered by caesarean section.*

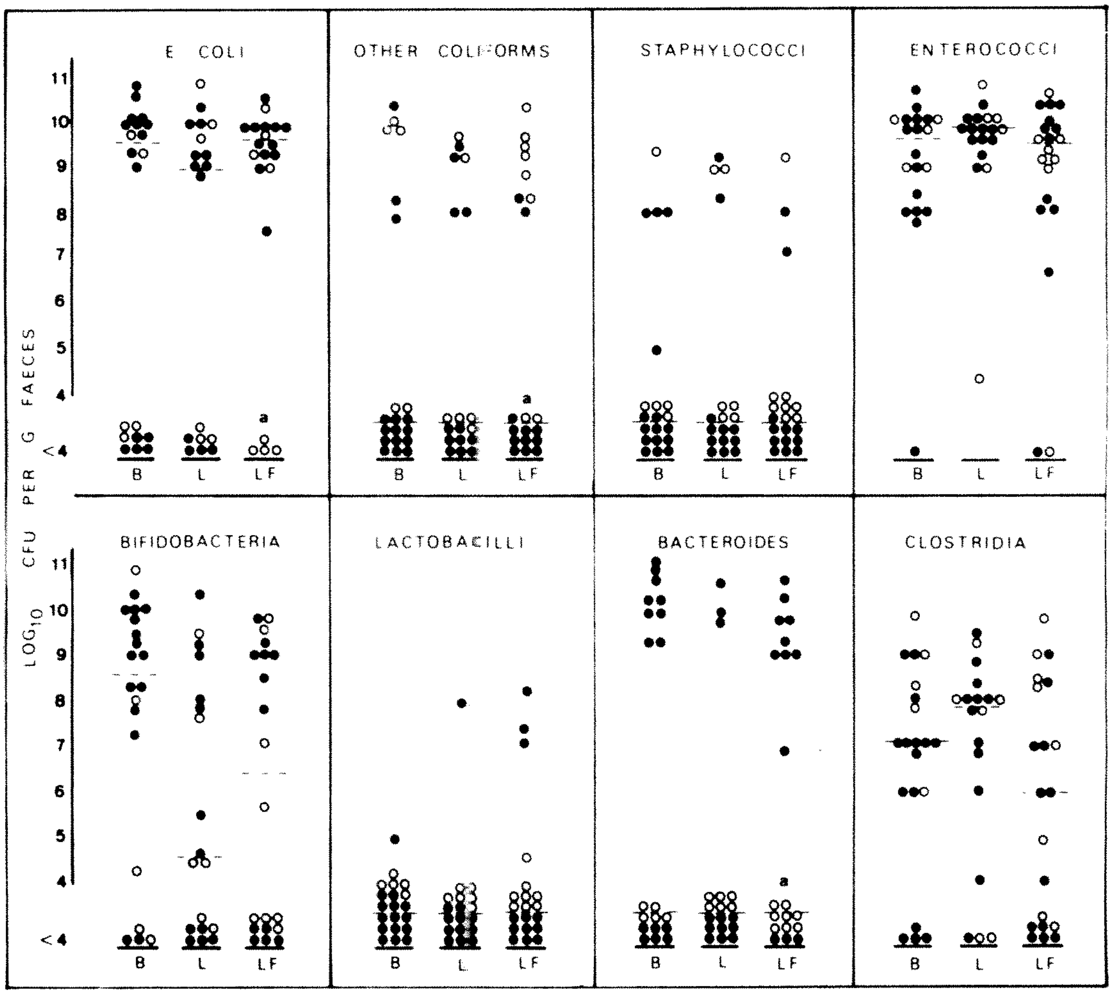

Fig 2 *Counts of individual organisms ($\log_{10}$ colony forming units (CFU) per g of faeces) in the three dietary groups (basic (B), L, and LF formulas) on day 14. Median count shown by horizontal broken line. a, Proportion of section delivered babies in whom an organism was not detected is significantly different (p<0·001) from vaginally delivered babies in the same dietary group (χ^2 with Yates's correction).* ● = *Babies delivered vaginally and* ○ = *babies delivered by caesarean section.*

χ^2 test (figs 1 and 2). (2) Patterns of dominance in individual *babies* (that is, a particular genus accounting for highest count in the bacterial population examined) analysed by the Fisher's exact test (fig 3).

Day 4

(1) *Counts of individual organisms (fig 1)*

(*a*) *Mode of delivery*—Differences were only apparent in the babies on formula LF. When comparing those babies born by caesarean section with those delivered vaginally (columns marked a in the figure) there were:

(i) fewer babies delivered by section who were colonised by *E coli* (nil compared with 100%, p<0·001) and bacteroides (nil compared with 58%, p<0·05).

(ii) more babies delivered by section who were colonised by other coliforms (62% compared with nil, p<0·001).

(*b*) *Dietary differences*—As the mode of delivery has caused differences, the effect of diet has been determined in the two groups separately. Results in the groups of babies on the basic and L formula groups were not different. The babies on formula LF differed from babies on the other two formulas in that there were more of them colonised with *E coli* and fewer with staphylococci (columns marked b and x in the figure).

(2) Patterns of dominance (fig 3)
No significant differences were detected.

Day 14
(1) *Counts of individual organisms (fig 2)*
(a) Mode of delivery—The differences in the babies on formula LF still persisted (columns marked a in the figure).
(b) Dietary differences—None were detected.

(2) *Patterns of dominance (fig 3)*
Bifidobacteria were the dominant organism in 25% of the groups on the basic formula compared with none of the group on formula LF (Fisher's exact test, p=0·02). The babies on formula L did not differ from those on formula LF. When the group on formula L was combined with the group on basic formula there was still an increased dominance of bifidobacteria compared with the group on formula LF (22% compared with nil, Fisher's exact test, p=0·03).

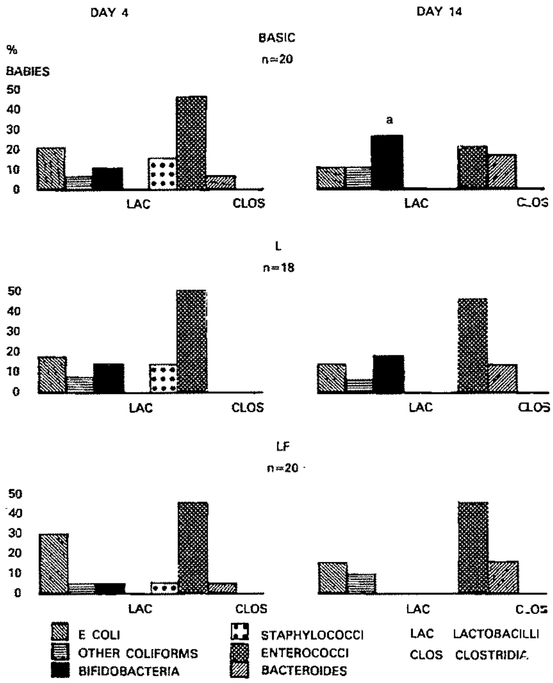

Fig 3 *Percentage of babies in whose faeces organisms were dominant on days 4 and 14; a=number of babies significantly different (p=0·02) from babies fed on formula LF (Fisher's exact test).*

Table 3 *Lactoferrin excretion in the faeces on day 11. Results are mg/day bovine lactoferrin*

	Formula		
	Basic (n=20)	*L (n=18)*	*LF (n=20)*
Range	0–2·2	2·4–7·1	1·9–6·0
Mean (SD)	0·55 (0·65)	4·50 (1·52)	3·67 (1·53)

FAECAL LACTOFERRIN CONCENTRATION
Table 3 shows the range of concentrations of lactoferrin in the faeces of all the babies. As expected the babies fed formulas containing lactoferrin (L and LF) excreted significantly more lactoferrin in their faeces than babies who were fed the basic formula (p<0·001). However, the observed excretion was substantially below the estimated average intake of babies weighing 3100 g (approximately 1300 mg daily). No correlation was found between the count of *E coli* in the infants' faeces on day 14 and the concentration of lactoferrin at day 11 regardless of whether they were fed a formula containing lactorferrin.

Discussion

If the lactoferrin was biologically active then according to the hypothesis we would expect (i) the addition of bovine lactoferrin to the basic formula (making it L formula) would have resulted in a change in the pattern of faecal flora in the direction of breast milk, and (ii) the addition of both lactoferrin and iron to the basic formula (to make LF formula) would reverse this trend, thus the flora of babies fed the basic and LF formulas would have a similar pattern.

These predictions based on the hypothesis were clearly not seen. The addition of lactoferrin to the basic formula had little or no effect upon the faecal flora as babies fed both basic and L formulas were similar. As lactoferrin was not associated with a movement of the pattern from the non-fortified basic formula *towards* that of the breast fed baby the second test of the hypothesis (that is, a reversal of the trend) really becomes redundant. In fact the addition of lactoferrin *and iron* did have a significant effect but not in the direction of a breast fed flora. At day 4 more babies on formula LF were colonised by *E coli* and fewer by staphylococci; at day 14 none of the babies on formula LF had dominant bifidobacteria.

Moreover only a minute amount of the ingested lactoferrin appeared intact in the faeces of the babies in our study; this suggests it had been broken down or degraded higher in the gut. Certainly the excretion of human lactoferrin by breast fed babies is reported in the range 5·7–23·6 mg/day, with the average faecal output amounting to only 1·1% of the lactoferrin ingested in the diet,[7] and our own observations (unpublished) are similar.

The ineffectiveness of the lactoferrin in vivo in this study could be due to one of three reasons: (a) the hypothesis based on the in vitro studies is not a reflection of what happens in babies, or (b) we used bovine lactoferrin and not human lactoferrin and this may have attracted foreign protein responses, or (c) other features of the system prevented the lactoferrin from being biologically active. In vitro studies have shown that bovine lactoferrin requires bovine antibodies (mainly IgG) and the complement cascade, lysozyme, and the presence of bicarbonate for it to be biologically active.[8-10] None of these factors were present in the formula. Generally therefore we favour explanations (b) and (c) for the lack of activity of the lactoferrin, but (a) cannot be excluded by the results of this study.

It is interesting that the addition of iron to the formula influenced the colonisation pattern, generally moving the faecal flora *away* from that seen in breast fed babies. Similar observations concerning iron fortification have been shown by Mevissen-Verhage *et al.*[11] This is of considerable potential importance both in our scientific understanding of factors affecting the gut and in infant feeding practice. We are currently examining the phenomenon further.

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References

[1] Balmer SE, Wharton BA. Diet and faecal flora in the newborn: breast milk and infant formula. *Arch Dis Child* 1989;**64:**1672–7.

[2] Balmer SE, Scott PH, Wharton BA. Diet and faecal flora in the newborn: casein and whey proteins. *Arch Dis Child* 1989;**64:** 1678–84.

[3] Archibald FS. Lactobacillus plantorum an organism not requiring iron. *Federation of European Microbiological Societies Letters* 1983;**19:**29–32.

[4] Dolby JM, Stephens S, Honour P. Bacteriostasis of E coli by milk II. Effect of bicarbonate and transferrin on the activity of infant feeds. *J Hyg (Camb)* 1977;**8:**235–42.

[5] Bullen JJ, Rogers HJ, Leigh L. Iron binding proteins in milk and resistance to Escherichia coli infection in infants. *Br Med J* 1972;**i:**69–75.

[6] Moreau MC, Thomasson M, Ducluzeau R, Raibaud P. Cinetique d'establissement de la microflora digestive chez le nouveau-ne humain en fonction de la nature du lait. *Reprod Nutr Dev* 1986;**26(2B):**745–53.

[7] Prentice A, Ewing G, Roberts SB, *et al.* The nutritional role of breast milk IgA and lactoferrin. *Acta Paediatr Scand* 1987;**76:** 592–8.

[8] Griffiths E, Humphreys J. Bacteriostatic effect of human milk and bovine colostrum on E coli: importance of bicarbonate. *Infect Immun* 1977;**15:**396–401.

[9] Spik G, Jorieux S, Mazurier J, Navarro J, Romond C, Montreuil J. Characterisation and biological role of human lactotransferrin complexes. In: Williams AF, Baum JD, eds. *Human milk banking.* Vevey: Nestlé Nutrition and New York: Raven Press, 1984:133–43.

[10] Reiter B, Brock JH, Steel ED. Inhibition of E coli by bovine colostrum and post colostral milk. II. The bacteriostatic effect of lactoferrin on a serum susceptible and serum resistant strain. *Immunology* 1975;**28:**83–95.

[11] Mevissen-Verhage EAE, Marcellis JH, Harmsen-van Amerongen WCM, de Vos NM, Berkel J, Verhoef J. Effect of iron on neonatal gut flora during the first week of life. *Eur J Clin Microbiol* 1985;**4:**14–8.

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Few food diets in the treatment of atopic eczema

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SUMMARY Sixty six children with severe atopic eczema were treated with highly restricted ('few food') diets followed, if they improved, by serial reintroduction of excluded foods. Twenty four patients (36%) improved considerably during the few food phase of the diet. Fifteen of these (23% of the study group) maintained this improvement on dietary treatment, of whom three abandoned the diet after periods ranging from six to 10 months, despite continued benefit, because they found the dietary restrictions too arduous. Thus 12 out of 66 children (18%) with severe eczema experienced prolonged and useful benefit from this dietary manoeuvre. Double blind food challenges performed in 10 patients failed to establish that parental identification of provoking foods is reliable. A search for historical and in vitro predictors of diet responsiveness was unsuccessful in this series.

A number of studies, both uncontrolled[1-3] and controlled,[4 5] have examined the therapeutic effect of 'simple' empirical diets excluding a few foods (cows' milk and egg in particular) in atopic eczema.

We are aware of seven published studies of highly restricted diets in the treatment of atopic eczema. Three of these deal with 'oligoantigenic' or 'few food' diets. Hathaway and Warner described problems of compliance in 40 children whose eczema improved on milk and egg exclusion (n=7), few food diets (n=30) or 'elemental' diets (n=3).[6] However, it is not clear what proportion of all those treated with diets, these 40 diet responsive children represent. Graham *et al* treated 26 children with a moderately restricted diet for two weeks followed by a few food diet for a final week.[7] Twenty two children completed the dietary protocol of whom 18 showed benefit, and 16 of these experienced some benefit from the few food phase of the diet. Armstrong *et al* used a similar protocol to assess a group of eczematous adults and children, starting with a simple exclusion diet followed, if improvement did not occur, by a few food diet.[8] Of 28 children who did not benefit from a simple exclusion diet, 12 proceeded to a few food diet and two of these 12 improved. The study by Juto *et al* deals exclusively with infants under 16 months and the effect of delayed sequential weaning in this group.[9]

The other three studies assessed the therapeutic effect of 'elemental' (theoretically antigen free) diets; one placebo controlled study in adults[10] showed no benefit while another open and uncontrolled study suggested improvement in five out of six adults on an elemental diet.[11] Hill and Lynch reported improvement in eight out of 10 eczematous children in an uncontrolled study of an elemental feed (Vivonex).[12]

No published study to date has examined the effect of few food diets in a large number of children with atopic eczema. We have used such diets to treat 66 children of varying ages with atopic eczema severe enough to justify such a disruptive measure. Our aims were: (i) to assess the therapeutic value of such diets, (ii) to identify the most frequent provoking foods by serial reintroduction, (iii) to confirm that improvement and deterioration in eczema was genuinely food related by administering double blind placebo controlled food challenges, (iv) to evaluate parental history, skin prick tests, and IgE antibodies as tests for the identification of provoking foods and, finally (v) to seek out any identifiable characteristics of diet responsive patients.

Patients and methods

Sixty six children (mean age 4·2 years, range 0·6–16·8 years; 36 boys) were selected from the dermatology outpatient clinic at the Hospital for Sick Children, Great Ormond Street during the period from late 1983 to early 1985. All had severe atopic eczema inadequately controlled by standard topical treatment and families who were judged to be capable of managing complex dietary manipulations. Sixty children (91%) had first degree relatives with atopic disease. Thirty two children were from social classes I and II, 26 from social class III, and eight from social classes IV and V. Twenty eight children (42%) had had at least one hospital admission for eczema. Fifty nine children (91%) were woken by itching on more than 50% of nights if the mean of the best and worst months of the latest six months was taken. Thirty five children (53%) had undergone previous dietary treatment for their eczema and in 13 this was said to have been beneficial. Thirty four children (52%) were already excluding one or more foods at the start of the study.

Parents were questioned as to the history of the disease in their child, observed dietary and environmental factors, and previous treatments. Where both the child's compliance and the severity of the eczema allowed, children were skin prick tested with extracts of cows' milk, egg, dog fur, cat fur, house dust mite, and Timothy grass pollen (Bencard). Blood was taken to measure circulating IgE antibodies using a modification of the radioallergosorbent test (RAST) technique described by Ceska *et al*[13] and antigens obtained from Sigma Chemical Co (ovalbumin and β lactoglobulin) and Bencard (house dust mite, cat fur, and Timothy grass pollen). Gastrointestinal permeability, a possible marker of food allergic disease,[14] was measured using the lactulose:rhamnose excretion ratio before and after the few food phase of the diet.

Each child was prescribed an individual few food diet after discussion with the parents. Dietary constituents were selected according to the following criteria: (i) foods generally regarded as commonly exacerbating atopic eczema were excluded, (ii) foods implicated on history in the exacerbation of the individual child's eczema were excluded, (iii) foods frequently eaten by the child were excluded, (iv) the diet was nutritionally adequate for the period concerned and, (v) the diet was judged by the parents, dietitian, and doctor to be as strict as the child could tolerate and palatable enough to ensure compliance. The diets therefore varied in strictness and constitution from child to child (diets A, B, and C in table 1 give an indication of the degree of this variation). The mean (SD) number of foods in the few food phase was 8·76 (3·76) with a range from one food (a casein hydrolysate for an infant aged 7 months) to 19 foods.

Eczema severity was assessed by a single clinician (MGP) before and after the few food phase of the diet. A modification of the visual score system used by Atherton *et al* was completed[4]: the body surface was divided into 20 areas (10 anterior and 10 posterior); for each area, the degree of redness, surface damage, and lichenification were assessed using firstly a numerical scale of severity (0 to 3) and, secondly, a figure expressing the percentage of the area concerned affected by the characteristic being assessed. These two figures were multiplied together and the products for all three characteristics for all 20 areas were added to give an overall score of severity. Twenty children had their visual eczema score assessed by one clinician (MGP) on two occasions not less than an hour apart as an indication of the variability of this measurement; the coefficient of variation calculated from these duplicate scores was 5·1%. Parents kept diary cards on which itch, redness, and sleep disturbance were each scored daily (0, 1, 2, 3 in order of increasing severity) and the amount of treatment used was documented. Dietary mistakes—that is excluded foods eaten in error—were also recorded on the diary card.

The median duration of the initial phase of the

Table 1 *Examples of the kind of diet prescribed*

A	*B*	*C*
Lamb	Rabbit	Venison Duck
Rice*	Potato	Rice* Potato and/or buckwheat, sago, yam
Casein hydrolysate†	Brassicas (cabbage, cauliflower, sprouts, broccoli)	Parsnips, carrots, cucumbers, courgettes
	Peaches, apricots, grapes, raisins	Dates, guava, lychee
	Olive oil	Sunflower oil
		Sugar

These examples indicate the range of few food diets used in order of decreasing strictness. Note frequent use of uncommon foods to which children were unlikely to have been previously exposed.
*Included additive and malt free 'rice krispies' produced by Kellogg Co Great Britain, Manchester.
†Nutramigen, Bristol-Myers.

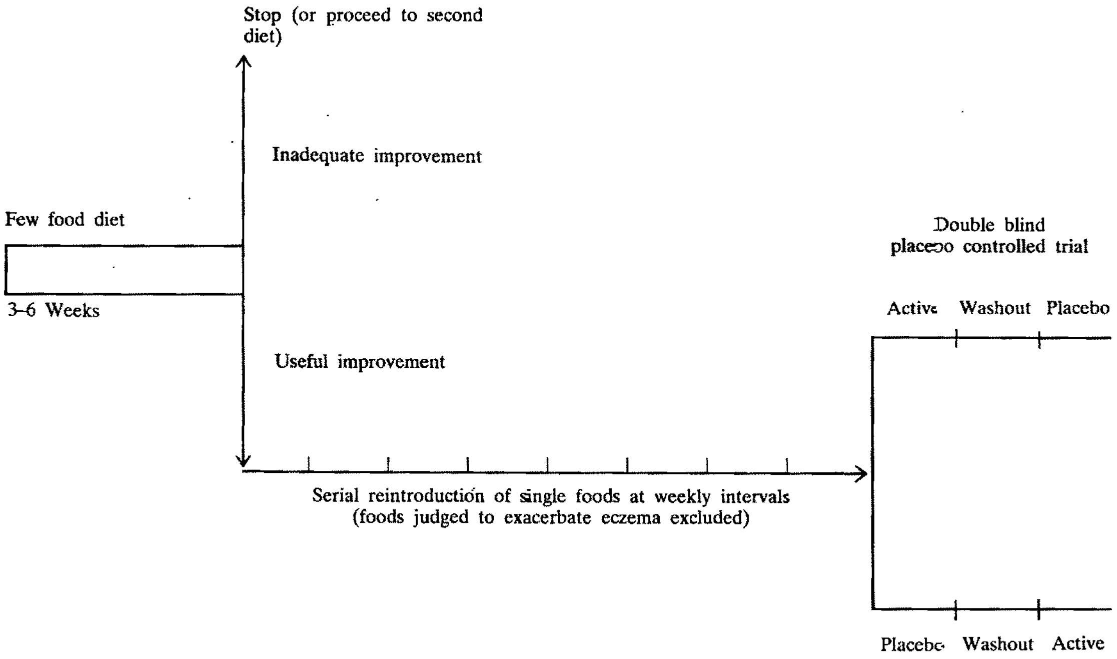
Stop (or proceed to second diet)
Inadequate improvement
Few food diet
3–6 Weeks
Useful improvement
Serial reintroduction of single foods at weekly intervals (foods judged to exacerbate eczema excluded)
Double blind placebo controlled trial
Active Washout Placebo
Placebo Washout Active

Fig 1 *Protocol for few food diet, serial reintroduction of foods and double blind placebo controlled food challenge.*

diet was 26 days (range 19–44 days, the longer duration resulting from a desire on some occasions to see whether a mild initial improvement would increase with time). At the end of this phase the doctor judged whether sufficient improvement had occurred to justify continuing dietary treatment (fig 1). This decision was a clinical one (to which visual score chart and diary card data were contributory but not fundamental) and judged whether improvement had occurred of such a degree as to justify continuing with what is a very difficult and protracted form of treatment.

Children who were not judged to have improved adequately either discontinued dietary treatment or proceeded to a second diet, similar in type but with entirely different constituents. Diet responsive children continued, with parental agreement, to the serial reintroduction of individual foods at weekly intervals. Foods being introduced were initially taken in small quantities to minimise the chance of an unexpected severe allergic reaction but from the second day in full helpings at least once a day. If the child's eczema remained apparently unaffected by the introduction of a food it was incorporated into the diet. If a deterioration occurred that progressed during exposure to the food and improved on its withdrawal, the food was judged to exacerbate the eczema and withdrawn. Sometimes a number of reintroductory and withdrawal periods were necessary to establish whether a food was exacerbating or innocuous.

In children who successfully completed the food reintroduction phase, double blind, placebo controlled food challenges were performed, (i) to confirm that the child's eczema improved and deteriorated in response to dietary manipulation and (ii) to validate parental identification of exacerbating foods.

A food identified by parents as exacerbating was disguised in a mixture with an innocuous food ('active') and the effect was compared with that of the innocuous food alone ('placebo'). Active and placebo components of the challenge were confirmed to be indistinguishable from one another by a group of 10 adult volunteers. Each mixture was taken daily in the same quantity and form, and for the same period as had been necessary to produce exacerbation on open introduction. When benzoic acid and tartrazine were tested, doses of 50 mg/day and 20 mg/day respectively (these doses being of the same order of magnitude as the daily intake of a normal child in the United Kingdom) were concealed in opaque capsules (Elanco). The order of active and placebo challenges was randomised and there was a one week wash out period between the active and the placebo periods.

Eczema was assessed by visual score chart before and after each challenge period and diary cards were kept by parents throughout the challenge period. Both parent and doctor indicated which they thought to be the exacerbating component before the mixtures were decoded.

Data from questionnaires, and from in vivo and in vitro testing, were evaluated as predictors of diet responsiveness.

Informed consent was obtained from all parents and approval for the study was obtained from the hospital's standing committee on ethical practice.

Results

OUTCOME OF FEW FOOD DIETARY TREATMENT (fig 2)

Forty three children performed one few food diet, 20 performed two, and two patients performed three. Children failed to complete the few food phase of the diet on five occasions; three of these were second diets in children who had already completed a first diet without benefit; one was a first diet in which chicken pox supervened and this child subsequently successfully completed a diet; one was a first diet in an infant who was cared for by an extended family in which it proved impossible to obtain compliance by all family members. Therefore 65 out of 66 children completed at least one few foo diet.

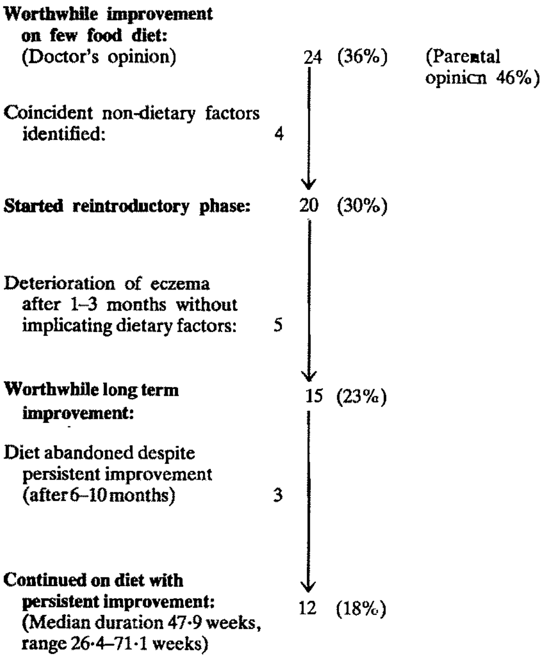
Worthwhile improvement on few food diet: (Doctor's opinion)
24 (36%)
(Parental opinion 46%)
Coincident non-dietary factors identified:
4
Started reintroductory phase:
20 (30%)
Deterioration of eczema after 1–3 months without implicating dietary factors:
5
Worthwhile long term improvement:
15 (23%)
Diet abandoned despite persistent improvement (after 6–10 months)
3
Continued on diet with persistent improvement: (Median duration 47·9 weeks, range 26·4–71·1 weeks)
12 (18%)

Fig 2 *Outcome of dietary treatment (number starting few food diet=66).*

Thirty children (46%) were felt by the parents t have improved sufficiently to justify continuing t food reintroduction, but the doctor concurred wit this opinion in only 24 cases (36%) (in no case whe the doctor thought sufficient improvement ha occurred did the parents disagree). Only in the 2 cases in whom the doctor judged sufficient improvement to have occurred was a reintroductory phas considered. In four of this group of 24, howeve coincident factors were thought by the doctor o parents, or both, to be important: two were admitted to hospital for intensive topical treatmen because of exacerbations of their eczema during pa of the few food phase of the diet and in two othe children a change of washing powder and season factors respectively were thought to have contributed significantly to the improvement.

Twenty children (30%) therefore proceeded t the reintroductory phase. In five of these 20 childre the eczema deteriorated during the first one to thre months of the reintroductory phase. Renewe exclusion of foods they had reintroduced produce no benefit; it was therefore not possible to ascrib their deterioration to dietary factors. These childre therefore withdrew from the study leaving 1 children (23%) who maintained worthwhile improvement on dietary treatment. Three of this grou of 15, however, abandoned dietary treatment despit persisting improvement after periods ranging fro six to 10 months. These children and their familie found the diets too burdensome despite thei beneficial effects. A review of the historical and tes data for these three children did not identify an distinguishing features; in particular they were no older than the children who persisted with dieta treatment.

Therefore, ultimately, 12 of 66 children (18% persisted with dietary treatment with long ter benefit. At the end of the study the mean duratio of dietary treatment in these children was 47· weeks (range 26·4–71·1 weeks).

OPEN REINTRODUCTION OF FOODS

The 15 children who improved on dietary treatment had reintroduced without deterioration mean of 15·3 foods each by the end of the stud (range 6–37). The overall rate of successful foo reintroduction was one food per three weeks (rang one food in seven weeks to one food in one and half weeks).

The foods most often judged to exacerbat atopic eczema on open reintroduction are listed i table 2.

Table 2 *Proportion of times individual foods reintroduced were identified as causing a deterioration in eczema*

Food	*Exacerbations/total reintroductions*
Meat/poultry/fish	
Lamb	2/11
Beef	6/13
Pork	3/13
Rabbit	0/3
Chicken*	6/12
Turkey	1/5
Cod*	4/8
Mackerel	1/1
Plaice	3/4
Milks and soya	
Cows' milk*	10/14
Goats' milk*	6/11
Sheeps' milk	1/5
Soya 'milk' formula*	5/7
Eggs	
Hens' egg*	6/9
Grains/yeast/malt	
Wheat*	7/13
Corn	5/12
Oats	4/13
Rice	1/8
Rye	0/2
Yeast	1/7
Malt	0/6
Vegetables	
Pea/runner bean	3/10
Carrot/parsnip	3/10
Brassicas	1/11
Tomato*	6/9
Onion/leek	3/5
Courgette/cucumber	0/1
Potato	1/8
Lentil	1/1
Fruit	
Apple/pear	3/13
Apricot/peach	1/10
Plum/nectarine	0/2
Grape/raisin	2/7
Orange*	6/11
Banana*	8/12
Pineapple	3/7
Blackcurrant	2/4
Melon	0/2
Additives	
Colourings/ preservatives	6/14
Other	
Sugar	0/7
Chocolate ('plain')	2/5
Tea	0/4

*Foods reintroduced by seven or more children and identified as exacerbating eczema on 50% or more of these reintroductions.

DOUBLE BLIND, PLACEBO CONTROLLED FOOD CHALLENGES

Ten of the 15 diet responsive patients completed double blind food challenges. The active and placebo components of these challenges are shown in table 3.

When the child had completed both active and placebo parts of the double blind challenge, parents and doctor each indicated which they thought had been the 'active' period; parents and doctor each identified the active period incorrectly on five occasions and correctly on five occasions. Parents and doctor were in agreement with each other in eight of these decisions.

Visual eczema scores were analysed both comparing the end of the active period with the end of the placebo period and also comparing the change from the beginning to the end of the active period with the analogous change in the placebo period. Diary card scores for itch, redness, sleep disturbance, steroid usage, and emollients were each analysed as follows: (1) the score for each variable for the whole of the active period was compared with the placebo period (paired *t* test). (2) The change in score for each variable from the start (first two days) to the finish (last two days) of the active period was compared with the same change in the placebo period (paired *t* test). There was no significant difference between active and placebo periods on any of these analyses.

During the interval between open introduction and double blind challenge there is a theoretical risk that the child's response to the food will alter. The mean interval from the identification of an exacerbating food on open introduction to double blind challenge was 45·4 days (range 16–181 days). There was no significant difference in the duration of this

Table 3 *Constituents of double blind food challenges (n=10)*

No of patients	*Active component*	*Placebo component*
4	Cows' milk	Goats' milk
2	Cows' milk	'Nutramigen'
1	Cows' milk	Soya 'milk'
2	Benzoic acid and tartrazine	Calcium gluconate
1	Benzoic acid	Calcium gluconate

interval between the double blind challenges in which the active component was correctly identified, whether by doctor or patient, and those in which it was incorrectly identified (p>0·01, Mann-Whitney U test in both analyses).

CHARACTERISTICS OF DIET RESPONSIVE PATIENTS

Data for the 15 diet responsive patients were compared with that for 41 unequivocally unresponsive patients. Excluded from this analysis were the child who did not complete a diet, the four children in whom concurrent non-dietary factors may have been responsible for the improvement, and the five children whose eczema deteriorated early in the reintroductory period without an apparent dietary cause. No significant differences were found with the exception of positive associations between response to diet and positive IgE antibody to ovalbumin, and larger number of 'slow' (occurring more than two hours after ingestion) cutaneous reactions to foods reported on history (*t* test, p<0·05 in both cases, table 4). The number of analyses performe was such that two results significant at the 5% leve would be expected to occur by chance.

Table 4 *Data compared in diet responsive and diet unresponsive patients*

Personal data:	Age. Sex. Age at onset of eczema.
	Severity of eczema (judged by visual score) at start of few food diet.
	Breast feeding (history of/duration of)
Environmental factors:	History of seasonal fluctuation of eczema.
	Presence of dog or cat in home with relevant skin prick test/IgE antibody positive.
	History of inhalent allergy.
Dietary factors:	Previous dietary treatment with or without benefit.
	Milk and egg already excluded at start of diet.
	History of food induced exacerbation of eczema.
	Number of reported 'immediate' (within two hours of ingestion) reactions to foods cutaneous and non-cutaneous).
	Number of reported 'slow' (more than two hours after ingestion) reactions to foods (cutaneous* and non-cutaneous).
	Reported reactions to non-foods (immediate and slow; cutaneous and non-cutaneous).
	Duration of few food phase of diet.
	Number of foods allowed in few food phase of diet.
In vivo and in vitro test data:	Skin prick test to foods/inhalants.
	Presence of IgE antibodies to: Ovalbumin* β lactoglobulin House dust mite Cat fur Timothy grass pollen
	Gastrointestinual permeability to oligosaccharides at start of diet.
	Change in gastrointestinal permeability with diet.

*Significant difference between diet responsive and diet unresponsive groups. (p<0·05).

PREDICTORS OF EXACERBATING FOODS

No useful assessment could be made of the predictive value of skin prick tests and IgE antibody test (RASTs) in the identification of exacerbating foods as the double blind challenge trial failed to validat the exacerbating effect of these foods.

VISUAL SCORE CHART AND DIARY CARD DATA

In the diet responsive group over the few food phas of the diet, the visual scores showed a mea improvement of 32·4% (range, 62·4% improvemen to 1·3% deterioration) and the cumulative diar card symptom score (itch, redness, and sleep disturbance combined) showed a mean improvemen of 20·9% (range, 57·9% improvement to 39·6% deterioration) if the first and last seven days of th few food phase of the diet were compared. Paradoxical deterioration in one or other measuremen occurred in three children; two showed an appreciable improvement in visual score but a deterioratio in symptom score; one child had a visual score tha was marginally worse (1·3%) with a 31% improvement in symptom score.

Topical steriod usage increased slightly and emollient use decreased during the few food phase, i neither case being significantly different from th changes in treatment in diet unresponsive patients

Discussion

These data suggest that few food diets are of limite benefit to children with severe atopic eczema Although it may be argued that our sample wa selected for diet unresponsiveness (as 54% continued to have severe eczema despite past dietar treatments), the response to the present dietar protocol was not in fact better in children who ha not previously experienced dietary treatment than i those who had (table 4).

Our few food diets were of varied composition bu excluded foods identified, from previous reports, a exacerbating eczema and observed as exacerbatin eczema in the individual child concerned. Comparison of the two outcome groups (diet responsive an diet unresponsive) does not suggest that, within th range of diets used in this study, extremely strict o prolonged diets are more effective than shorter les strict diets (table 4).

Of particular interest in the present study was th identification of a group of four children whos improvement could be ascribed to non-dietar factors and a further five children who deteriorate in the first one to three months after improving on the few food diet without dietary factors appearing to be responsible for their deterioration. These children, if they had been classified as diet responsive, would have increased our diet responsive group by 38%; clearly careful review of coincident non-dietary factors and follow up to confirm that improvement is maintained should be part of any future dietary study of this type.

Three children in the group judged to have shown considerable improvement in fact showed a deterioration in one of the indices of severity, in the presence of an appreciable improvement in the other. These cases emphasise the difficulty of quantifying the severity of this condition. Diary card symptom scores may be affected by extraneous factors (for example, sleep disturbance due to intercurrent illness), and both the weight allocated to individual symptoms and the periods at the beginning and end of the few foods phase of the diet for which cumulative scores are calculated (in this study seven days) are arbitrary. Visual scores at the time of consultation may not invariably reflect a symptomatic improvement—long standing lichenification is unlikely to change measurably over three weeks and erythema may be exacerbated transiently by ambient irritants, temperature, or emotion. For these reasons decisions as to dietary outcome on the basis of preset numerical criteria may misallocate patients.

The poor response rate in this study meant that our double blind trial was small. Furthermore, the interval between open reintroduction and double blind challenge was long in some cases and theoretically an alteration of the effect of the active food (or indeed of the placebo) might have occurred during this interval. However, the results as they stand have two implications. Firstly, parental identification of provoking foods in atopic eczema is unreliable, presumably because of the fluctuating nature of the disease and the multiplicity of confounding influences (environmental, emotional, etc.). Secondly, the results do not demonstrate exacerbation of these children's eczema by foods. Therefore, by implication, the improvement observed, on an open uncontrolled basis during the few food phase of the diet, cannot be ascribed with any confidence to food exclusion.

It is of interest that, though analysed differently, the double blind trial described in the study of Armstrong *et al* also showed correct identification of the active component in only half of the challenges.[8] In this context, Wilson and Silverman's demonstration that asthmatic children may respond to allergenic foods, not by simple bronchospasm, but by an increase in bronchial lability rendering them more susceptible to a second agent, may be relevant.[15] Should a similar two stage mechanism occur in atopic eczema, simple provocation tests of the kind used in these studies would not necessarily identify it.

The list of foods implicated on open introduction (table 2) therefore remains unvalidated. The similarity to the foods implicated by the patients in Hathaway and Warner's study[6] and in the study of Armstrong *et al*[8] is of note, however, as is the frequent implication of soya which, though used as a placebo agent in milk and egg exclusion studies in the past,[4 5] may now be viewed as an unsatisfactory choice for such a role.

Numerous comparisons of historical and test data for diet responsive and diet unresponsive patients showed only two weak associations with diet responsiveness; however, as stated above, the number of analyses performed was such that two results significant at the 5% level would be expected to occur by chance. Neither association would have usefully improved the response rate if used as the criterion for dietary treatment in our study group. Of particular interest was the absence of any association between increased intestinal permeability and response to diet and the absence of a significant change in permeability over the dietary period in children whose eczema improved considerably on dietary treatment; though increased permeability has been shown to occur in childhood atopic eczema[16] and in association with food induced hypersensitivity reactions of probable IgE mediated type,[14] it does not appear to usefully predict response to exclusion diets of the type we have assessed.

'Simple' empirical diets such as that studied by Atherton *et al* are a well established part of the treatment of childhood atopic eczema.[4] Further work is needed to clarify the role of elemental diets in a controlled fashion in larger numbers of children with extreme eczema than have been studied hitherto. Given the burden, both emotional and financial, of the few food diet, the eagerness (well illustrated in our study) of parents to pursue such diets even when the benefits are marginal, and our failure to achieve even a small response group validated by double blind challenge, it would seem prudent to restrict the use of such diets unless and until further evidence is forthcoming.

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References

[1] Talbot FB. Eczema in childhood. *Med Clin North Am* 1918;1: 985–96.

[2] Rowe A, Rowe AH. Atopic dermatitis in infants and children. *J Pediatr* 1951;**39**:80–6.

[3] Hammar H. Provocation with cows' milk and cereals in atopic dermatitis. *Acta Derm Venereol (Stockh)* 1977;**57**:159–63.

[4] Atherton DJ, Sewell M, Soothill JF, Wells RS, Chilvers CED. A double-blind controlled crossover trial of an antigen avoidance diet in atopic eczema. *Lancet* 1978;i:401–3.

[5] Neild VS, Marsden RA, Bailes JA. A double-blind crossover trial of egg and milk exclusion diets in atopic eczema. *Br J Dermatol* 1984;**114**:117–23.

[6] Hathaway MJ, Warner JO. Compliance problems in the dietary management of eczema. *Arch Dis Child* 1983;**58**:463–6.

[7] Graham P, Hall-Smith SP, Harris JM, Price ML. A study of hypoallergenic diets and sodium cromoglycate in the management of atopic eczema. *Br J Dermatol* 1984;**110**:457–67.

[8] Armstrong D, Neild VS, Bailes JA, Marsden RA. Exclusion diets in atopic eczema. In: Chandra RK, ed. *Food allergy*. St John's, Newfoundland: Nutrition Research Education Foundation, 1987:267–72.

[9] Juto P, Engberg S, Winberg J. Treatment of infantile atopic dermatitis with a strict elimination diet. *Clin Allergy* 1978;**8**: 493–500.

[10] Munkvard M, Danielsen L, Hoj L, *et al.* Antigen-free diet in adult patients with atopic dermatitis. *Acta Derm Venereol (Stockh)* 1984;**64**:524–8.

[11] Villaveces JW, Heiner DG. Experience with an elemental diet (Vivonex R). *Ann Allergy* 1985;**55**:783–9.

[12] Hill DJ, Lynch BC. Elemental diet in the management of severe eczema in childhood. *Clin Allergy* 1982;**12**:313–5.

[13] Ceska M, Eriksson R, Varga JM. Radioimmunosorbent assay of allergens. *J Allergy Clin Immunol* 1972;**49**:1–9.

[14] Andre C. Diagnosis of food allergy and evaluation of therapeutic efficacy by measurement of intestinal permeability. *Presse Med* 1986;**15**:105–8.

[15] Wilson N, Silverman M. Diagnosis of food sensitivity in childhood asthma. *J R Soc Med* 1985;**78**(suppl 5):11–6.

[16] Pike M, Heddle RJ, Boulton P, Turner AW, Atherton DJ. Increased intestinal permeability in atopic eczema. *J Invest Dermatol* 1986;**86**:101–4.

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Type II hyperprolinaemia in a pedigree of Irish travellers (nomads)

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SUMMARY We describe a study of 312 subjects in 71 families near related to a proband with type II hyperprolinaemia. The subjects were Irish travellers (nomads) among whom consanguineous marriage and high fertility are common. Thirteen additional cases of type II hyperprolinaemia were discovered; all were offspring of consanguineous unions. A further 50 subjects were found to have mild hyperprolinaemia. We found a strong association between type II hyperprolinaemia and seizures during childhood but no significant association with mental handicap. Most adults with type II hyperprolinaemia enjoyed normal health and there was no evidence that maternal hyperprolinaemia compromised fetal development. The documented association between type II hyperprolinaemia and seizures may be related to the neuromodulatory or reducing-oxidising effects of proline and pyrroline-5-carboxylate, respectively, that has been shown in vitro. Alternatively, another genetic defect closely linked to the type II hyperprolinaemia allele could be the explanation.

Hyperprolinaemia is a disorder of metabolism characterised biochemically by a raised concentration of plasma proline and by aminoaciduria consisting of proline, hydroxyproline, and glycine. The urinary excretion of hydroxyproline and glycine is due to a saturation by proline of an active transport system in the renal tubule, which shares affinity for proline, hydroxyproline, and glycine.[1 2] Proline and hydroxyproline are non-essential amino acids. Beyond early infancy there is virtually no proline in normal urine.

Twenty families with the rare biochemical disorder, hyperprolinaemia, are known to the authors from the literature.[1 3] On the basis of the findings in these families and pedigrees the hyperprolinaemia trait can be divided into two forms: type I hyperprolinaemia, caused by defective proline oxidase activity and type II hyperprolinaemia, caused by deficient Δ-pyrroline-5-carboxylic acid dehydrogenase (P5CD) activity. Both forms are the result of mutant alleles at different genetic loci and are inherited in autosomal recessive fashion. Consanguinity of the parents was noted in several of the 20 previously reported families.

Metabolism of L-proline and clinical phenotypes

L-proline is first oxidised by proline oxidase to Δ-pyrroline-5-carboxylic acid (P5C). P5C is in equilibrium spontaneously with glutamic acid γ semialdehyde, which is oxidised by P5CD to glutamic acid. Glutamic acid semialdehyde can also be transaminated to form ornithine. Proline oxidase is mitochondrial and found in relatively few tissues while P5CD is cytosolic and ubiquitous.[4] The pathway of mammalian proline metabolism is shown in fig 1.

Based on a number of reports the normal plasma proline range may lie between 80 and 350 μmol/l.[1 5] The concentration in type II hyperprolinaemia, with few exceptions, is in excess of 1300 μmol/l and may be as high as 3000 μmol/l, whereas, in type I the concentration rarely exceeds 1700 μmol/l. As the block in type II hyperprolinaemia is at the second stage of catabolism, people with this condition have appreciably raised concentrations of plasma P5C and excrete P5C in their urine. These latter findings distinguish type II hyperprolinaemia from type I.

Proline has been found to have neurotransmitter

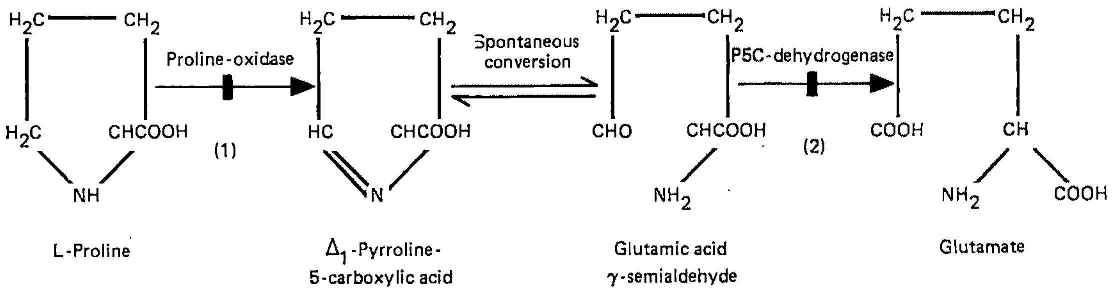

Fig 1 *Pathway of mammalian proline metabolism with two known human metabolic blocks indicated: (1) in type I hyperprolinaemia, (2) in type II hyperprolinaemia.*

properties to which P5C may contribute but the effect of high plasma proline or high plasma P5C concentrations on brain function is unclear.[1 6] In the earliest reports of type I and type II hyperprolinaemia an association of the conditions with diverse clinical abnormalities was reported, namely, hereditary nephropathy, deafness, mental handicap, and seizures.[1 5 7] In subsequent reports, families were described in which hyperprolinaemia occurred in the absence of clinical disease and many believe that the hyperprolinaemias are benign conditions.[8-12] However, a cause and effect relationship between type II hyperprolinaemia and convulsive seizures was not completely ruled out.

The investigation

The investigation reported here commenced with the family of the proband, who had hyperprolinaemia, iminoglycinuria, and mental handicap and was subject to seizures. Part of the family pedigree is shown in fig 2; the proband is IV.22. There was a high incidence of seizures among his siblings, four of whom were mentally handicapped. Subsequently, the investigation was extended to many other families with the same paternal ancestry. All were 'travellers' (itinerants, tinkers, gypsies) among whom teenage marriage of cousins, particularly first cousins, and high fertility are common.[13] The word 'gypsies' is used in the descriptive sense only. Members of the group are not of Romany origin but are racially Irish.[14] Most of those over 20 years of age are illiterate. In recent years many have settled in houses. While not of Romany origin their characteristics and practices resemble, in many respects, those described by Thomas for Romany gypsies of the United States among whom marriage between second cousins is considered ideal.[15] Travellers are very suspicious of outsiders. However, the first named author is generally trusted by them having done voluntary social work with them for many years. Thomas got exposure to their culture by caring for them and gaining the title 'Gypsy doctor'.

Samples of urine and blood were collected in the family house or caravan, apart from a few collected during clinic or hospital attendance. Cooperation, although sometimes slowly attained, was excellent there being only two refusals at initial approach. However, requests for repeat specimens were sometimes unsuccessful. Furthermore, the scattered geographic location and mobility, even to the United Kingdom, were limiting factors in obtaining specimens for follow up tests. During the repeated home visits the family medical history was taken, with detailed questioning about possible convulsive episodes. Confirmation of a history of seizures given by the subjects or their parents was obtained from family doctors and hospital records. The term seizure embraces febrile convulsions, isolated single seizures, recurrent petit mal and grand mal epileptic type convulsions. Despite the hardship and rigours of their way of life travellers have no special health problems other than an increased liability to physical injury.[13 14]

Assessment of IQ (by the revised Wechsler intelligence scale for children) was performed on 19 schoolgoing children of the pedigree: five with type II hyperprolinaemia, five with mild hyperprolinaemia, and nine with normal plasma proline. One of the children with type II hyperprolinaemia had an IQ of 55 and another an IQ of 70. One child with mild hyperprolinaemia had an IQ of 75, and the remainder had an IQ of 80 or higher. Mental handicap is defined as having an IQ <70 on the Wechsler scale. Two preschool children with type II disease, who showed normal development, were not formally tested. Other members of the pedigree

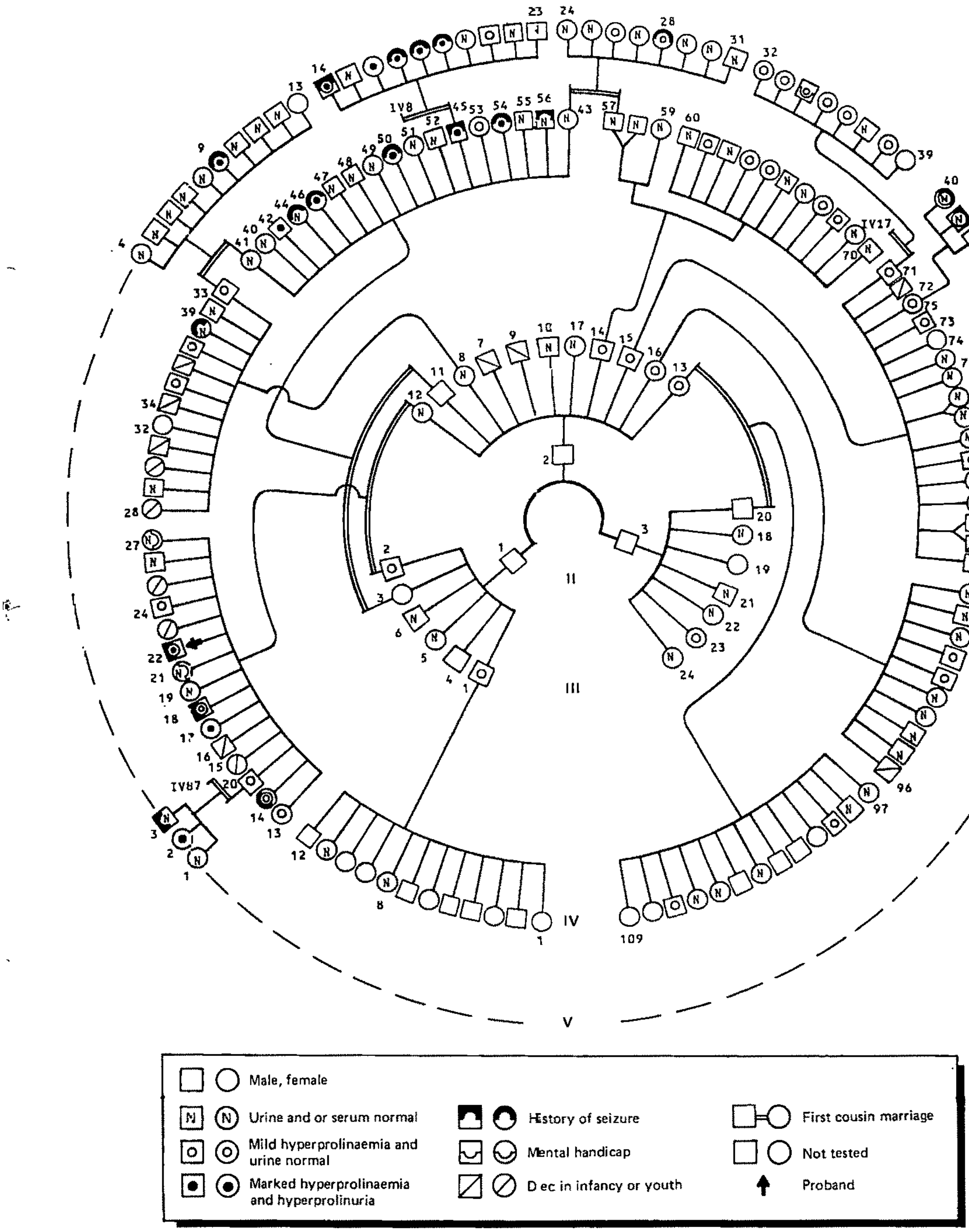

Fig 2 *Type II hyperprolinaemia in a pedigree of Irish travellers.*

with mental handicap were known from previous clinical assessments.

Methods

Preliminary screening involved the qualitative testing of urine from the family of the proband and some close relatives for amino acids by two dimensional paper chromatography. Those showing prolinuria, their immediate family, and some members of the extended family, had blood samples taken in plain tubes, and their serum or plasma proline concentrations were determined on a Locarte amino acid analyser. A total of 312 subjects were screened: urine was collected from 280 and blood from 167. In 32 cases no urine was collected but a blood sample was. Many repeat samples were taken. Concentrations of urinary amino acids were determined on the Locarte analyser using random samples. The reference values for serum proline used for adults were those given by Dickenson *et al*: mean 184 (range 102–336) µmol/l.[16] The reference values of Clayton *et al* were used for children: mean 185 (range 89–281) µmol/l.[17]

The method described by Strecker was used in testing for orthoaminobenzaldehyde reactive substances in the urine.[18]

Plasma P5C concentration and leucocyte P5CD activity were quantitated on five subjects with prolinuria and hyperprolinaemia and 32 other pedigree members and two normal controls. For plasma P5C concentrations, 10 ml of heparinised whole blood was immediately chilled in an ice slurry. Plasma obtained by centrifugation was deproteinised with 50% trichloroacetic acid to a final concentration of 10% and stored at −20°C. Plasma P5C concentrations were determined by a published method.[19] For P5CD measurements, leucocytes were obtained by dextran sedimentation from 10 ml of heparinised whole blood. The cells were washed twice in phosphate buffer (0·1 M, pH 8·0) and stored at −20°C. The samples were carried to Bethesda, Maryland in carbon dioxide snow. P5CD activities were measured using a previously described specific radioisotopic method,[20] and proteins in the extracts were estimated by the method of Lowry *et al.*[21]

Results

In addition to the proband, who was discovered to have hyperprolinaemia in 1972, at 10 years of age, prolinuria was found in a further 13 subjects (three males, 10 females) from five families. Serum proline concentration was above 2000 µmol/l in 10 and slightly under that level in two (table 1). It was not convenient to take blood from one child (V.19) but quantitative urinalysis showed iminoglycinuria.

Table 1 *Personal details and amino acid profiles of pedigree members with type II hyperprolinaemia*

Pedigree No (fig 2)	*Sex*	*Age (years)*	*Qualitative urinary proline*	*Quantitative urinary amino acids (µmol/l)*			*Serum proline (µmol/l)*		*Plasma P5C (µmol/l)*	*Suffered from seizures*	*Mental handicap*
				Proline	*Hydroxyproline*	*Glycine*	*Fasting*	*Non-fasting*			
Adults:											
IV.17	F	31	Positive	42 100	763	19 673	1818	2047	14·75	No	No
IV.22*	M	28	Positive	29 500	518	5 934	2663	2218	18·87	Grand mal	Severe
IV.42	M	36	Positive	14 211	641	3 664	2257	2178	Not tested	No	No
IV.45	M	31	Positive	22 101	1145	7 606	2673	2277	Not tested	Grand mal	No
IV.46	F	29	Positive	25 585	548	7 050	2075	2371	22·13	Petit mal	No
IV.50	F	22	Positive	40 897	4171	20 591	2182	1951	Not tested	Grand mal	No
IV.54	F	18	Positive	25 357	1082	6 119	2124	1278	29·57	Grand mal	No
Reference values				Nil	5–20	169–1298		102–336			
Children:											
V.2	F	2	Positive	8 718	947	3368		1193 1762	Not tested	No	No
V.9	F	10	Positive	6 793	107	1347		2283	23·35	Grand mal	Slow
V.14	M	12	Positive	37 352	3769	15 052		1821	Not tested	Grand mal	Mild
V.16	F	10	Positive	40 215	2177	11 156		2478	Not tested	No	No
V.17	F	9	Positive	20 572	1447	7721		2210	Not tested	Grand mal	No
V.18	F	7	Positive	2 102	84	1603		2713	Not tested	Grand mal	No
V.19	F	5	Positive	8 279	230	3412		Not tested	Not tested	Grand mal	No
Reference values				Nil	2–22	165–1420	105–261	89–261			

*Proband.
Samples of urine were random.

Fifty other subjects (27 males, 23 females) had a serum proline concentration higher than the reference values. These are classified as having mild hyperprolinaemia. None of the 50 was found to have prolinuria. The proline concentration in seven was over 700 μmol/l, being above the venous plasma concentration of 0·8 mM (695 μmol/l) at which prolinuria occurs according to Scriver *et al.*[2] Quantitative urinalysis on one girl (V.28) with a serum concentration of 1278 μmol/l (confirmed on repeat test) showed no aminoaciduria. This girl and 15 others with mild hyperprolinaemia had normal concentrations of plasma P5C and had P5CD present in their peripheral leucocytes. The serum concentration in the 147 subjects tested is given in table 2. With a single exception proline was the only raised serum amino acid. A mentally handicapped boy (V.34), with multiple congenital defects, had mild hyperprolinaemia (546 μmol/l) and mild alaninaemia (555 μmol/l). There was no apparent difference between fasting and non-fasting serum proline concentrations.

It proved convenient to test urine from nine of the 14 with prolinuria for orthoaminobenzaldehyde reactive substances. The findings ranged from 0·053 to 1·677 mean optical density value. Urine from 20 control (general hospital) patients gave a mean optical density value of 0·01325 with a range 0·00 to 0·027.

Tests for P5CD in peripheral leucocytes showed that it was undetectable in the five subjects with hyperprolinaemia and prolinuria who were tested. Their plasma P5C concentrations were high at 14·75; 18·87; 22·13; 23·35, and 29·57 μmol/l. The range in 32 pedigree members with normal plasma proline or mild hyperprolinaemia was 0·128 to 1·71 μmol/l. Two non-pedigree controls had concentrations of 1·41 and 1·2 μmol/l, respectively. All these 34 subjects showed the presence of P5CD in their peripheral leucocytes ranging from 6·83 nmol/hour/mg to 52·4 nmol/hour/mg. Summaries of the numbers tested, results, and findings relating to seizures and mental handicap are given in fig 3. No significant association was shown between mental handicap and proline status. There was, however, a highly significant association between the occurrence of seizures and proline status when analysed by the χ^2 test: 78·39, $p<0·01$. The excess of seizures in those with raised proline was accounted for entirely by the group with type II hyperprolinaemia. There was no association between mild hyperprolinaemia and seizures.

Table 2 *Serum proline concentrations in the 147 members of the pedigree tested*

Proline (μmol/l)	*No of subjects*	*Proline (μmol/l)*	*No of subjects*
0–100	1	1700–1800	1*
>100–200	26	>1800–1900	1*
>200–300	48	>1900–2000	0
>300–400	26	>2000–2100	2*
>400–500	14	>2100–2200	2*
>500–600	8	>2200–2300	3*
>600–700	4	>2300–2400	1*
>700–800	5	2500–2600	2*
>800–900	1	2700–2800	1*
1200–1300	1		
Subtotal	134	Subtotal	13

*Indicates prolinuria.

With the object of establishing a contrast with the pedigree of the proband, the homes and caravans of all other non-pedigree travellers located in the same general area, to a total of 41 families with 259 children and young adults, were visited and medical histories taken. First cousin marriage was less common than in the proband pedigree. While not near related to the pedigree, some would have common ancestors. There were three (1·2%) with a history of recurrent seizures and none had an isolated single seizure. The pedigree families and the non-pedigree families were not matched for age and sex but were of generally similar structure. Just three of the non-pedigree travellers had serum proline estimated, all being normal.

FAMILY PEDIGREES AND CLINICAL FINDINGS

Among the pedigree sampled there were 71 marriages. Only three of these were not between first to third degree cousins. Families containing one or more members with hyperprolinaemia or other significant finding are set out in fig 2. Family members are numbered sequentially in birth order by generation. Because of the large size of completed families and widespread marriage of teenage first cousins only key family units of the pedigree are depicted.

The proband, who suffered from severe mental handicap and recurrent seizures, was one of 11 surviving children of 15 born live to a consanguineous union (family of III.2 and III.12). The father (III.2) had mild hyperprolinaemia. Five of the 20 pregnancies of the mother (III.12) ended in miscarriage and there were four deaths in infancy or childhood. Of the 11 survivors, five had severe mental handicap and physical defects. Of these five one, the proband, had appreciable hyperprolinaemia and two had mild hyperprolinaemia; these three were also subject to seizures, as were two who died in childhood. Of the six other

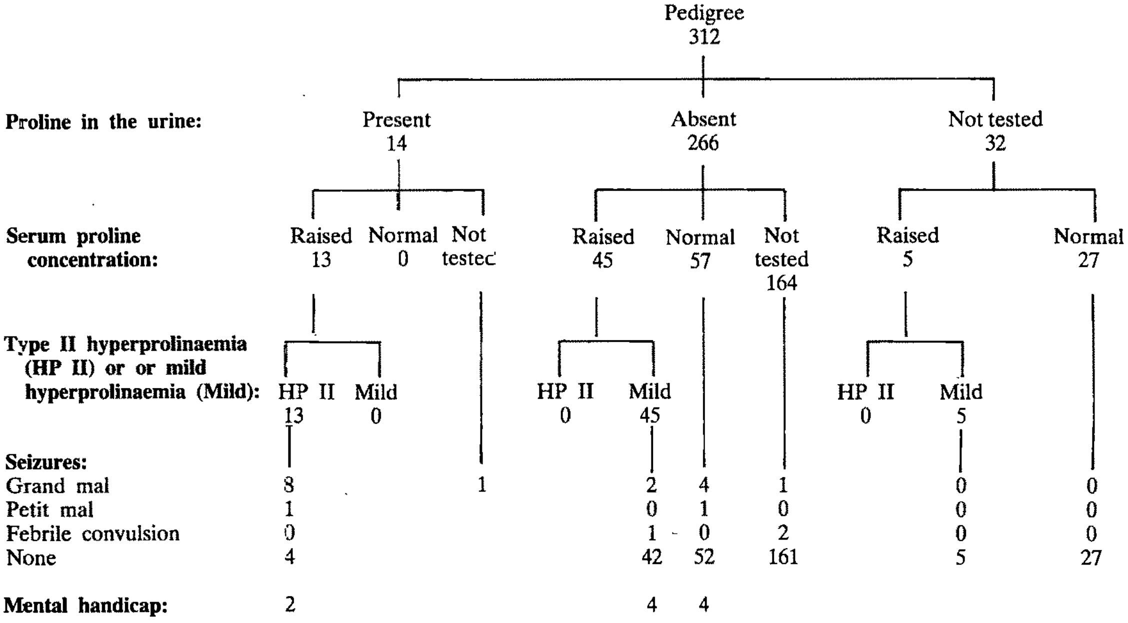

Fig 3 *Presence of proline in urine and serum proline concentrations in members of the pedigree, showing the numbers having seizures and mental handicap.*

survivors, five were generally healthy, one of whom (IV.17) had type II hyperprolinaemia and three had mild hyperprolinaemia (IV.13, 20, 24). Subject IV.17 suffered from occasional episodes of depression requiring hospitalisation but had no history of seizures. Six of her eight children had mild hyperprolinaemia but none had aminoaciduria (a newborn infant was not tested). The high morbidity and mortality in the offspring of III.2 and III.12 is exceptional for travellers.

The second most significant family of the pedigree was the 17 live born and surviving offspring of subject III.11 who was dead at the time of the survey, and of III.3 who declined sampling. This family were double first cousins of the proband's family. None had mental handicap. Five had type II hyperprolinaemia (IV.42, 45, 46, 50, and 54), four of whom gave a history of seizures in childhood. One of these (IV.45) had 10 children, five of whom had type II hyperprolinaemia, four with a history of recurrent seizures and one with mild mental handicap. Subject IV.41 with normal serum proline, had one child (V.9) with type II hyperprolinaemia, who had recurrent seizures and was a slow learner. Another member of the family (IV.43) had one child (V.28) who had mild hyperprolinaemia to a level of 1278 µmol/l, normal urine, normal plasma P5C and P5CD present in peripheral leucocytes. This child had had a single childhood febrile convulsion and was a slow learner. Two other members of the family of III.11 and III.3, who had normal proline profiles, had had recurrent seizures which ceased in IV.44 at 22 years of age and was controlled in the other (IV.56) who was in his early teens. One of the four children of IV.48 had two febrile convulsions. The child's urine was normal. Plasma proline was not estimated. The children of IV.48 are not shown in fig 2.

One other consanguineous couple had two children (V.40 and 41) with severe mental handicap and epilepsy since early childhood. Both had a normal urine and serum amino acid profile. The elder child died at 9 years of age subsequent to being studied. A third recently born child appeared normal. Their mother (IV.75) had mild hyperprolinaemia. One young woman (IV.38) developed grand mal seizures in late teenage. The second generation ancestor (II.2) suffered from traumatic epilepsy for five years before his death at 54 years of age.

In the remainder of the extended pedigree (not shown in fig 2) there was one child with spina bifida who had occasional seizures, two who had single febrile convulsions, and one with multiple congenital defects. Other than subject II.2, all the persons referred to are the offspring of teenage marriages of first cousins.

In addition to those of the proband, hospital records or discharge reports were available for eight of those with type II hyperprolinaemia in respect of 30 admissions because of seizures and fever ranging up to 41°C. Upper respiratory tract infection, sometimes associated with vomiting, was the most common positive finding, with no evidence of major underlying pathology. There were three episodes of diarrhoea and meningism with negative results on lumbar puncture. Electroencephalographic reports were available for four pedigree children. Three with type II hyperprolinaemia gave a normal reading, but subject IV.56, with a normal proline profile and who was on maintenance anticonvulsants, indicated a convulsive tendency.

MATERNAL HYPERPROLINAEMIA

Four women with type II hyperprolinaemia had teenage marriages. Subject IV.17 had eight children, IV.46 had five children, IV.50 had three children, and IV.54 had two children. With a single exception, the children were physically and developmentally normal. The exception was the third child of IV.17. He was the child with mild hyperprolinaemia, alaninaemia, and multiple congenital defects referred to earlier (V.34). The mother of this family (IV.17), who suffered occasionally from depression, had a pulmonary embolus six days after her sixth confinement. A further confinement three years later was uneventful but her eighth confinement, after another six years, was followed by pulmonary embolus 10 days after the birth. Thus 17 of 18 children born to mothers with type II hyperprolinaemia appeared physically and developmentally normal. Five of the 10 children of one man with type II hyperprolinaemia (IV.45) had type II hyperprolinaemia, four had had seizures and one had mild mental handicap. The remaining five children were apparently healthy. Another man with type II hyperprolinaemia (IV.42) had eight healthy children (not shown in fig 2).

Discussion

The biochemical findings in 14 members of this pedigree fulfilled the established criteria for type II hyperprolinaemia, namely, appreciable hyperprolinaemia, iminoglycinuria, and orthoaminobenzaldehyde reactive substances in the urine. Unfortunately, it was not feasible to assay for leucocyte P5CD activity and plasma P5C in the entire pedigree because of the nomadic life style of some and unwillingness for further sampling in others. But in five affected and 31 other members of the pedigree in which P5CD and P5C assays were done, the initial diagnosis was confirmed in every case. In agreement with findings in previously reported pedigrees, proline was the sole raised serum amino acid. The unusual, if not unique, association of hyperglycinaemia with hyperprolinaemia reported by Pavone *et al* was not found in this series.[11]

An unexpected finding in this pedigree is the high number of subjects with mild hyperprolinaemia (approximately one third) for which we have no simple explanation. For type II hyperprolinaemia, Scriver *et al* outlined a straightforward association between phenotype and presumed genotype in this metabolic disorder with autosomal recessive inheritance.[1] Probands and siblings with hyperprolinaemia exceeding 1300 μmol/l are homozygotes. Heterozygotes with intermediate activities of P5CD have normal serum proline concentrations. In the current pedigree the transmission of type II hyperprolinaemia, as represented by the 14 presumed homozygotes, is clearly autosomal recessive. Furthermore, the finding of normal plasma proline concentrations in eight children of one man with type II hyperprolinaemia (IV.42), all expected heterozygotes at the P5CD locus, is consistent with the idea that heterozygotes have decreased P5CD activities but do not have abnormalities in plasma proline. At first sight, the finding of mild hyperprolinaemia in six out of seven children tested from a union between a mildly hyperprolinaemic man (IV.71) and a woman with type II hyperprolinaemia (IV.17) may suggest a causal link between P5CD deficiency and mild hyperprolinaemia. Mild hyperprolinaemia occurred in the face of normal leucocyte P5CD activities, however, and normal concentrations of plasma P5C. Taken together, these findings suggest that mild hyperprolinaemia and P5CD deficiency are not associated. The physiological or biochemical basis for mild hyperprolinaemia remains unelucidated.

It is in the larger context of the association, if any, of clinical pathology to the metabolic abnormalities in hyperprolinaemia that this pedigree is of special interest. Only one of the seven previously reported probands was identified by screening. In all the others type II hyperprolinaemia was found by diagnostic studies for a clinical disorder—for example, seizures. Thus an association between clinical symptoms and hyperprolinaemia could not be made because of biased ascertainment. Moreover, the identification of asymptomatic siblings with type II hyperprolinaemia in certain pedigrees argues against a direct link to clinical symptoms. Consequently, most investigators consider type II hyperprolinaemia a benign disorder.[1 9 11 12]

In the present series, diagnostic studies in pursuit of a causative factor for physical and mental disability applied only to the proband. The other 13 subjects with type II hyperprolinaemia were discovered during the survey of the proband's extended family. Six of the seven adults among the 14 cases of type II hyperprolinaemia are physically healthy and pursued a normal life. The seventh adult was severely handicapped mentally and suffered from recurrent seizures. Furthermore, fertility and fetal development were apparently normal as 18 children were born to four women with type II hyperprolinaemia. The one child with congenital defects and also the adult with severe mental handicap probably were chance occurrences. Thus findings in this pedigree generally support the prevalent belief that type II hyperprolinaemia is a benign condition.[1 9 11 12]

Nevertheless, there is a striking association between type II hyperprolinaemia and childhood seizures, at least in this pedigree. Four of the seven adults had recurrent seizures in childhood. Five of the seven children had a history of recurrent seizures, often associated with fever, up to the age of 7 years. The severity of these episodes was attested by the necessity for their hospitalisation and treatment with anticonvulsants. Importantly, there is no association of mild hyperprolinaemia and seizures.

It could be argued that the observed association of seizures with type II hyperprolinaemia in this pedigree was related to the unusual lifestyle of the traveller population. Thus we surveyed a population to serve as controls. With few exceptions all travellers available within the catchment area were surveyed to minimise selection bias. There were 24 persons (4·2%) with a history of seizures among the 571 pedigree and non-pedigree travellers studied. This percentage is similar to that reported by Annegers *et al* from Rochester, Minnesota, where the incidence of any type of seizure through age 20 years was 4.1%.[22] When the occurrence of seizures in the pedigree travellers is compared with that in the non-pedigree travellers a significant difference is shown ($\chi^2=9{\cdot}58$, $p<0{\cdot}01$). In the pedigree group, 16 of 21 with seizures were among the 64 with raised serum proline or prolinuria and the excess over the expected in those with raised proline is accounted for entirely by the group with type II hyperprolinaemia (fig 3).

What remains, therefore, is the explanation for this documented association of seizures with type II hyperprolinaemia in this pedigree. One possible explanation is that linkage occurs at the genetic rather than at the enzymatic level—that is, the predisposition to seizures is unrelated to the metabolic consequences of absent P5CD activity. Instead, some other gene responsible for the inherited tendency to seizures may be closely linked to the gene for P5CD. Although there is no evidence supporting this hypothetical linkage, it cannot be ruled out.

A much more likely explanation is that the predisposition to seizures is a consequence of the metabolic derangement caused by the absence of P5CD activity. Several of these derangements have been shown by direct measurement in type II hyperprolinaemia including raised plasma and, presumably, cellular concentrations of proline and pyrroline-5-carboxalate. Certainly, proline itself has been found to be a neuromodulator,[6] and specific high affinity uptake mechanisms for proline regulated by neuropeptides have been characterised in brain synaptosomes.[23]

In addition, pyrroline-5-carboxylate, a constituent of normal plasma,[19] is a potent effector in transferring reducing-oxidising potential into cells.[24 25] Its uptake is mediated by a specific carrier mechanism that transfers oxidising potential pari passu cell entry.[26] Due to this transfer of oxidising potential, P5C appreciably effects glucose metabolism by stimulating the activity of the pentose phosphate shunt.[24–26] Importantly, cultured human glioma cells are very sensitive to this effect of P5C.[27] It is tempting to speculate that these effects of proline and P5C, which have been shown in vitro, are related to the predisposition to seizures. In the case of P5C, its perturbation of glucose metabolism if superimposed on hypoglycaemic and febrile episodes, both common occurrences in childhood, may be especially relevant in the association of seizures with type II hyperprolinaemia.

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References

[1] Scriver CR, Smith RJ, Phang JM. Disorders of proline and hydroxyproline metabolism. In: Stanbury JB, Wyngaarden JB, Fredickson DS, Goldstein JL, Brown MS, eds. *The metabolic basis of inherited diseases.* 5th ed. New York: McGraw-Hill, 1983.

[2] Scriver CR, Efron ML, Schafer IA. Renal tubular transport of proline, hydroxyproline and glycine in health and in familial hyperprolinemia. *J Clin Invest* 1964;**43**:374–85.

[3] Whelan DT, Connor WT. Maternal hyperprolinaemia. *Lancet* 1980;**ii**:981.

[4] Adams E, Frank A. Metabolism of prolines and the hydroxyprolines. *Annu Rev Biochem* 1980;**49**:1005–61.

[5] Schafer IA, Scriver CR, Efron ML. Familial hyperprolinemia, cerebral dysfunction and renal anomalies occurring in a family with hereditary nephropathy and deafness. *N Engl J Med* 1962;**267**:51–60.

[6] Felix D, Kunzle H. Iontophoretic and autoradiographic studies on the role of proline in nervous transmission. *Pflugers Arch* 1974;**350**:135–44.

[7] Perry TL, Hardwick DF, Lowry RB, Hanson S. Hyperprolinaemia in two successive generations of a North American family. *Ann Hum Genet* 1968;**31**:401–7.

[8] Simila S, Visakorpi JK. Hyperprolinaemia without renal disease. *Acta Paediatr Scand [Suppl]* 1967;**177**:122.

[9] Mollica F, Pavone L, Antener I. Pure familial hyperprolinemia: isolated inborn error of aminoacid metabolism without other anomalies in a Sicilian family. *Pediatrics* 1971;**48**:225–31.

[10] Sardharwalla IB, Hill A. Infantile hyperprolinaemia. *Arch Dis Child* 1975;**50**:745.

[11] Pavone L, Mollica F, Levy HL. Asymptomatic type II hyperprolinaemia associated with hyperglycinaemia in three sibs. *Arch Dis Child* 1975;**50**:637–41.

[12] Mollica F, Pavone L. Hyperprolinaemia: a disease which does not need treatment? *Acta Paediatr Scand* 1976;**65**:206–8.

[13] Flynn MP. Mortality, morbidity and marital features of travellers in the Irish midlands. *Ir Med J* 1986;**79**:308–10.

[14] Anonymous. *Report of the Travelling People Review Body.* Dublin: The Stationery Office, 1983.

[15] Thomas JD. Gypsies and American medical care. *Ann Intern Med* 1985;**102**:842–5.

[16] Dickenson JC, Rosenblum H, Hamilton PB. Ion exchange chromatography of the free aminoacids in the plasma of the newborn infant. *Pediatrics* 1965;**36**:2–13.

[17] Clayton BE, Jenkins P, Round JM. *Paediatric chemical pathology: clinical tests and reference ranges.* Oxford: Blackwell Scientific Publications, 1980.

[18] Strecker JD. The interconversion of glutamic acid and proline. The formation of Δ-pyrroline-5-carboxylic acid from glutamic acid in Escherichia coli. *J Biol Chem* 1957;**225**:825–34.

[19] Fleming GA, Hagedon CH, Granger AS, Phang JM. Pyrroline-5-carboxylate in human plasma. *Metabolism* 1984;**33**:739–42.

[20] Valle D, Goodman SI, Applegart DA, Shih VE, Phang JM. Δ-pyrroline-5-carboxylic acid dehydrogenase deficiency in cultured skin fibroblasts and circulating lymphocytes: *J Clin Invest* 1976;**58**:598–603.

[21] Lowry OH, Rosbrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951;**193**:265–75.

[22] Annegers JF, Hauser WA, Anderson VE, Kurlund LT. The risks of seizure disorders among relatives of patients with childhood onset epilepsy. *Neurology* 1982;**32**:174–9.

[23] Rhoads DE, Samkaram H, Raghupathy E. Selective inhibition of synaptosomal proline uptake by leucine and methionine enkephalins. *J Biol Chem* 1983;**258**:12233–7.

[24] Phang JM, Downing SJ, Yeh GC, Smith RJ, Williams JA. Stimulation of the hexose-monophosphate pentose pathway by Δ^1-pyrroline-5-carboxylate in cultured cells. *J Cell Physiol* 1982;**110**:255–61.

[25] Yeh GC, Phang JM. Stimulation of phosphoribosyl pyrophosphate and purine nucleotide production by pyrroline-5-carboxylate in human erythrocytes. *J Biol Chem* 1988;**263**:13083–9.

[26] Mixson AJ, Phang JM. The uptake of pyrroline-5-carboxylate: group translocation mediating the transfer of reducing-oxidising potential. *J Biol Chem* 1988;**263**:10720–4.

[27] Loreck DJ, Galarraga J, Van der Feen J, Phang JM, Smith BH, Cummins CJ. Regulation of the pentose phosphate pathway in human astrocytes and gliomas. *Metabolic Brain Disease* 1987;**2**:31–46.

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Changes in leg length and height during treatment with somatropin

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SUMMARY Knemometry has been used in a short term double blind placebo controlled trial in 13 patients with normal variant short stature receiving treatment with somatropin to assess the power of the change in lower leg velocity at one month to predict the increase in height velocity at six months. Used in this way the method has a positive predictive value and sensitivity of 90% and a negative predictive value and specificity of 50%. Although not a perfect discriminatory test, knemometry is a more reliable and less invasive way of analysing the likely value of a growth promoting treatment than metabolic assays in individual patients. Given the possible future rapid expansion of the use of somatropin in short stature of various aetiologies there is a need for a relatively simple and inexpensive means of evaluating response to treatment.

Knemometry provides a precise and reproducible means of measuring short term changes in lower leg length and is capable of detecting daily or weekly changes in lower leg length velocity.[1 2] It is not possible to extrapolate changes in lower leg length directly to changes in stature because of the inherent variability of growth in lower leg length, which proceeds in a series of lulls and spurts,[3] and to use the growth of a group of bones to mirror the growth of the entire skeleton is conceptually imprecise. Nevertheless we have previously shown (using knemometry) in patients with Turner's syndrome that failure to respond to somatrem during a four week period predicts failure to respond to a six month course of somatrem in the same dose assessed by auxology.[4] The positive predictive power of knemometry was weaker in this model because apparent responses may have been the result of coincidental 'mini growth spurts'.

The possibility of a placebo effect of injections or of increased hospital attendance has never been satisfactorily excluded in trials of growth hormone treatment. A placebo controlled trial in the United Kingdom was in progress at the time of withdrawal of pituitary growth hormone in 1985 and no other placebo controlled study has ever been undertaken.[5]

Pituitary gigantism is the experiment of nature that testifies that any normal child can be made to grow faster and achieve a greater adult height if given sufficient growth hormone. Normal variant short stature is one of several terms used to describe short children who are growing slowly but who have peak plasma growth hormone responses to conventional testing of >15 U/l. This may represent the lower extreme of physiological growth hormone secretion in a range extending from classical growth hormone deficiency through short normal to tall normal children,[6] although not all workers have found the association between stature and physiological growth hormone profiles to be as straightforward as was initially proposed.[7 8]

The dose response of normal variant short stature to treatment with growth hormone is not well defined. Previous reports indicate that about two thirds of such patients respond to a conventional dose of growth hormone of 2U/day, but whether more would respond if the dose were larger is unknown.[9 10]

At present the identification of benefit from any growth promoting treatment requires conventional auxology for at least six months and is usually defined as an increase in height velocity of >2 cm/year. It should be remembered that benefit from growth hormone treatment in terms of increase in final adult height has not yet been shown apart from in patients with 'classical' growth hormone deficiency. If lack of response to a treatment protocol could be determined over a shorter period than six months (as seems to be the case in Turner's syndrome[4]) then an ineffective, expensive, and uncomfortable treatment could be withdrawn or modified with consequent benefit to the patient and financial saving.

Table 1 *Patients details at entry to trial. The patients are ranked according to eventual increase in height velocity*

Case No	*Sex*	*Age (years)*	*Bone age at start of treatment (years)*	*Height (SD score)*	*Height velocity (SD score)*	*Target height* (SD score)*
1	Female	4·1	2·8	−1·9	−1·6	−0·7
2	Male	11·7	8·8	−4·6	−3·8	−1·4
3	Male	10·8	9·3	−2·5	−1·4	Adopted
4	Male	4·5	3·5	−2·9	−1·9	+0·3
5	Male	12·7	9·2	−3·0	−1·6	−1·5
6	Male	13·2	10·6	−2·3	−3·0	−1·2
7	Female	11·1	8·8	−3·1	−0·7	−0·4
8	Male	5·1	3·8	−2·8	−2·2	+1·3
9	Male	10·3	10·3	−2·3	−0·5	0·0
10	Male	5·1	2·4	−4·4	−2·0	−1·5
11	Male	13·6	11·8	−2·5	−4·5	−0·5
12	Male	11·7	8·6	−4·2	−0·8	−2·9
13	Female	10·8	8·5	−2·2	−1·9	−0·2

*Target height=(height of mother+height of father)/2 where parental height is adjusted by adding 13 cm to the mothers' height in the case of a boy and subtracting 13 cm from fathers' height in the case of a girl.

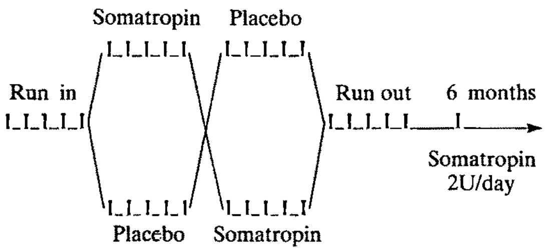

Figure *Design of trial: weekly measurements for a minimum of 16 weeks followed by monthly measurements for six months. Somatropin was given in doses of 0·5 ml or 2 U/day.*

Patients and methods

Thirteen children with short stature (more than 2 SD below normal height for age) and a poor growth rate (≤25th centile height velocity for chronological age) determined by accurate auxology for at least a year were recruited from patients referred to this hospital. Where it was possible to calculate the target height from the heights of the parent all were apparently failing to fulfill their genetic height potential. All had documented adequate plasma growth hormone concentrations in response to various provocation tests as defined by the former Health Services Human Growth Hormone Committee.[11] All subjects were healthy and prepubertal by clinical criteria at the beginning and end of the study, and had a starting bone age of less than 11 years for girls and 12 years for boys. Informed consent for participation in the trial was obtained from the children and parents, and the study was approved by the local ethics committee. Patient details at entry are given in table 1.

The design of the trial is shown in the figure. The subjects were measured weekly for a minimum of 13 weeks. There was at least four weeks' 'run in' before two four week double blind periods of injection with placebo or somatropin before a four week 'run out' period. The placebo and somatropin were provided as identically packaged lyophilised powder by Kabi-Vitrum AB, Stockholm, who held the code until the end of the trial. The patients received 0·5 ml placebo, or the same volume, equivalent to 2U somatropin, allocated at random, by daily subcutaneous injection. After the 16 week period of intensive measurement, the patients attended monthly for six months during which they received 2U somatropin daily.

At each visit four estimations were made of lower leg length and height, sitting height and weight were measured as previously described.[2] The subjects attended for measurement on the same weekday at the same time of afternoon to correspond with the reported plateau of postural lower leg compression and kept daily activity as uniform as possible.[12] Any changes in routine, or illnesses since the previous visit, were recorded and an enquiry was made at each visit for possible side effects or problems with treatment. A specimen of urine was tested for glucose at each visit and glycated haemoglobin was also measured at the end of the trial to detect clinically relevant glucose intolerance. A radiograph for the estimation of TW2 bone maturation index was taken at the start and the end of the study.[13]

The design of the trial permitted determination of: (i) The ability of knemometry to detect active growth promoting treatment by an acceleration in lower leg velocity. If there was no short term response this could be *either* a failure of treatment (as shown subsequently from analysis of height velocity) *or* a failure of the method. (ii) Detection of a short term placebo effect of injections. (iii) The power of short term lower leg velocity changes to predict subsequent clinically significant height response to treatment with growth hormone defined conventionally as an increase in height velocity of more than 2 cm/year.

After all the patients had completed the trial, rates of lower leg growth during the intensive measurement phase were calculated by linear regression through the median lower leg estimation, as described previously.[2] Unlike height velocity, positive lower leg response was not defined numerically. The higher lower leg velocity was used to predict the active phase of treatment and the analysis sent to KabiVitrum. Only then was the code broken and the actual treatment order established. The predictive power of knemometry was calculated by comparing short term positive and negative responses with the positive and negative responses in the six month height velocity.[14]

There were no defaulters at any planned visit and the injections were well tolerated. One child withdrew from the study after the intensive period of measurement and her data are not included in the analysis of predictive power of knemometry.

Results

There was no significant difference between lower leg growth during the run in and run out phases, and so these have been combined as a 'no treatment' velocity (N) and compared with velocity during placebo (P) or active treatment (S) in table 2. The following patterns of response were seen: S>P>N=5; S>N>P=4; S>P=N=2; in other words knemometry was able to detect the active component in 11 of 13 subjects. In one child N>S>P and in another P>N>S. There was no detectable placebo effect in that P was greater than N on six occasions, equal on two occasions, and less than N on four occasions. There was no association of treatment order S-P or P-S to magnitude of lower leg response, and thus no delayed treatment effect of somatropin on lower leg growth.

As there was no placebo effect, a mean was taken of all lower leg velocities during periods without active treatment (P+N1+N2/3) and the value was subtracted from the lower leg velocity when taking somatropin (S) to show the change that may be seen in patients with normal variant short stature.

The bone age gap (chonological age−bone age) and height velocity of each child at the end of the study and the changes in these two measurements as

Table 2 *Lower leg velocity (mm/week) during the three phases of intensive measurement, all phases when growth hormone was not given combined, and the difference between lower leg velocities with and without short term treatment with growth hormone*

Case No	*Active treatment (S)*	*Placebo (P)*	*No treatment (N)*	*All phases when growth hormone was not given combined (P+N1+N2)/3*	*Difference between lower leg velocities with and without short term treatment with growth hormone*
1	1·18	0·49	0·41	0·43	+0·75
2	0·69	0·44	0·24	0·30	+0·39
3	0·54	0·21	0·32	0·28	+0·26
4	1·54	0·38	0·34	0·35	+1·19
5	0·67	0·05	0·28	0·20	+0·47
6	0·68	0·26	0·33	0·31	+0·37
7	0·88	0·46	0·29	0·34	+0·54
8	1·26	0·15	0·58	0·43	+0·83
9	0·96	0·22	0·22	0·22	+0·74
10	0·39	0·31	0·35	0·34	+0·05
11	0·18	0·52	0·24	0·33	−0·15
12	0·52	0·34	0·21	0·25	+0·27
13	0·38	0·12	0·50	0·37	+0·01

Table 3 *Patients' ages at entry to trial; chronological/bone age gaps and height velocities at start and finish of trial, and changes in these values*

Case No	Age (years)	Before trial		After trial		Change	
		Chronological/bone age gap (years)	*Height velocity (cm/year)*	*Chronological/bone age gap (years)*	*Height velocity (cm/year)*	*Chronological/bone age gap (years)*	*Height velocity (cm/year)*
1	4·1	1·3	5·9	1·7	12·5	+0·4	+6·7
2	11·7	2·9	2·1	3·4	6·7	+0·5	+4·6
3	10·8	1·5	4·1	1·7	8·4	+0·2	+4·3
4	4·5	1·0	5·4	1·5	9·6	+0·5	+4·2
5	12·7	3·5	4·1	3·7	7·7	+0·2	+3·6
6	13·2	2·6	4·1	2·6	7·6	0·0	+3·5
7	11·1	2·3	4·7	2·4	8·1	+0·2	+3·4
8	5·1	1·3	4·7	1·4	7·6	+0·1	+2·9
9	10·3	0·0	3·6	−0·2	6·2	−0·2	+2·6
10	5·1	2·7	4·7	3·0	7·2	+0·3	+2·5
11	13·6	1·8	3·5	2·0	4·8	+0·2	+1·3
12	11·7	3·1	4·5	3·3	5·8	+0·2	+1·3
13	10·8	2·3	4·0	Withdrew			

a result of treatment with somatropin are shown in table 3. All 12 subjects remaining in the trial showed an increased height velocity (mean +3·4 cm/year; range +1·3 to +6·7), but this was not deemed to be clinically relevant in two children who did not achieve the previously defined increase of +2 cm/year.[15] There was a significant increase of the bone age gap in the group as a whole (p=<0·01) and hence an improved adult height prediction in eight of 10 responders to somatropin. There was no correlation between six month height velocity response to age, peak growth hormone response to stimulation tests, previous height SD score, previous height or lower leg velocity, and dose of somatropin. There was a weak and not significant (r=0·48) linear correlaton between increase in height velocity and magnitude of lower leg response. No side effects of treatment were reported, nor was there any glycosuria. Glycated haemoglobin concentrations at the end of the trial were within the normal range in all cases.

Comparing the concordance of short term and long term reponses shows that nine subjects responded in both the short term and the long term, one subject did not respond in either the short term or the long term, but more importantly there were two subjects who were discordant. Of the two discordant children one showed no apparent increase in lower leg velocity but did have an increased height velocity of 2·4 cm/year, a false negative response. Knemometry had a 90% positive predictive value and 90% sensitivity for the response to treatment with somatropin in patients with normal variant short stature judged conventionally, and a 50% negative predictive value and specificity. The numbers studied (nine of 10 and one of two, respectively) are too few to warrant extrapolation of these percentages.

Discussion

Knemometry is a powerful method for examining short term changes in lower leg growth rate. Wit *et al* have shown that lower leg growth of normal children in two successive six week periods varied significantly in 5 of 31 cases, and that to be confident at a 5% level that a change in lower leg growth was not a chance association with a growth spurt, a change in lower leg velocity of >0·37 mm/week was necessary.[16] Our trial of the predictive power of knemometry in Turner's syndrome confirmed that a short term growth response had only a 50% predictive value for a six month response, but had an excellent negative predictive value of 100%.[4] This led to the proposal that knemometry could be used to screen children who would not respond to a long term therapeutic growth promoting regimen.

In patients with Turner's syndrome we showed a clear bimodal lower leg response to short term somatrem, with a range of short term lack of response of −0·55 to +0·14, and of response from 0·31 to 0·67 mm/week. In this study we chose not to extrapolate from the results of the trial in patients with Turner's syndrome, or from the conclusions of Wit *et al* to preset a definition of what constituted a short term response in patients with normal variant short stature, but took the greater lower leg velocity on treatment as predictive of the active component and used the subsequent height velocity response to determine whether we could set a threshold of lower leg response that could be used as a screening test in future studies.

The double blind placebo controlled design of this study provided a more rigorous means of testing this proposal in these patients, who are likely to provide the greatest future demand for treatment with somatropin. We have confirmed that there is no placebo response to treatment that is detectable by knemometry, and that 2 U/day somatropin increased lower leg velocity by 0·25 mm/week or more in 10 of 13 subjects. The results of the present study differ from those of our study in patients with Turner's syndrome in that most patients responded to somatropin in the long term, and there was an example of a false negative short term response. Analysis of the predictive power of knemometry using the results of both studies gives a positive predictive value of 72%, a negative predictive value of 86%, a sensitivity of 93% and specificity of 55%.

Knemometry is at least as accurate as measurement of metabolic variables such as nitrogen retention,[17] assay of insulin like growth factor 1,[18–21] and growth hormone profile analysis[8] in predicting response to treatment with somatropin. Assay of procollagen III related peptides measures the short term metabolic consequence of active growth, but is likely to show exactly the same variability as knemometry.[22] Although there is a weak correlation for the group as a whole, the inherent variability of lower leg growth means that it is not possible to predict the magnitude of later height velocity response for an individual child, and that prediction of a good response to treatment is less certain than prediction of poor response. Although not ideal, we consider that there is a place for this technique in the evaluation of growth promoting regimens.

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References

[1] Wales JKH, Milner RDG. Variation in lower leg growth with alternate day steroid treatment. *Arch Dis Child* 1988;**63**:981–3.

[2] Wales JKH, Milner RDG. Knemometry in assessment of linear growth. *Arch Dis Child* 1987;**62**:166–71.

[3] Hermanussen M, Geiger-Benoit K, Burmeister J, Sippel WG. Periodical changes of short term growth velocity ('mini growth spurts') in human growth. *Ann Hum Biol* 1988;**15**:103–9.

[4] Wales JKH, Milner RDG. Knemometry as a predictor of response to somatrem in Turner's syndrome. *Acta Paediat Scand* 1987;**337**(suppl):37–9.

[5] Buchanan CR, Law CM, Milner RDG. Growth hormone i short, slowly growing children and those with Turner's syndrome. *Arch Dis Child* 1987;**62**:912–6.

[6] Albertsson-Wikland K, Isaksson O, Rosberg S, Westphal O Secretory pattern of growth hormone in children of differen growth rates. *Acta Endocrinol* 1983;**103**(suppl 256):72.

[7] Rose SR, Ross JL, Uriarte M, Barnes KM, Cassorla FG, Cutle GB. The advantage of measuring stimulated as compared with spontaneous growth hormone levels in the diagnosis of growth hormone deficiency. *N Engl J Med* 1988;**319**:201–7.

[8] Costin G, Kaufman FR. Growth hormone secretory patterns i children with short stature. *J Pediatr* 1987;**110**:362–8.

[9] Van Vliet G, Styne DM, Kaplan SL, Grumbach MM. Growth hormone treatment for short stature. *N Engl J Me* 1983;**309**:1016–22.

[10] Raiti S, Kaplan SL, Van Vliet G, Moore WV. Short-term treatment of short stature and subnormal growth rate with huma growth hormone. *J Pediatr* 1987;**110**:357–61.

[11] Milner RDG, Burns EC. Investigation of suspected growth hormone deficiency. *Arch Dis Child* 1982;**57**:944–7.

[12] Valk IM, Langhout Chabloz AME, van Gilst W. Intradail variation of the human lower leg length and short term growth—a longitudinal study in fourteen children. *Growth* 1983;**47** 397–402.

[13] Tanner JM, Whitehouse RH, Marshall WA, Healy MJR, Goldstein H. *Assessment of skeletal maturity and prediction of adu height. (TW2 method)*. London: Academic Press, 1975.

[14] Vecchio TJ. Predictive value of a single diagnostic test i unselected populations. *N Engl J Med* 1966;**274**:1171–3.

[15] Milner RDG, Russell-Fraser T, Brook CGD, *et al*. Experienc with human growth hormone in Great Britain: the report of th MRC Working Party. *Clin Endocrinol* 1979;**11**:15–38.

[16] Wit JM, van Kalsbeek EJ, van Wijk-Hoek JM, Leppink GJ Assessment of the usefulness of weekly knemometric measurements in growth studies. *Acta Paediatr Scand* 1987;**76**:974–80

[17] Wright JC, Brasel JA, Aceto T, *et al*. Studies with huma growth hormone (HGH). *Am J Med* 1965;**38**:499–516.

[18] Grunt JA, Howard CP, Daughaday WH. Comparison of growth and somatomedin C responses following growth hormone treatment in children with small-for-dates short stature, significan idiopathic short stature and hypopituitarism. *Acta Endocrinc (Copenh)* 1984;**106**:168–74.

[19] Rudman D, Moffit SD, Fernhoff PM, McKenzie WJ, Kenn JM, Bain RP. The relation between growth velocity and serur somatomedin C concentration. *Journal of Endocrinology an Metabolism* 1981;**52**:622–7.

[20] Raiti S, Moore WV, Van Vliet G, Kaplan SL. Growth stimulating effects of human growth hormone therapy i patients with Turner's syndrome. *J Pediatr* 1986;**109**:944–9.

[21] Gertner JM, Genel M, Gianfredi SP, *et al*. Prospective clinic trial of human growth hormone in short children without growt hormone deficiency. *J Pediatr* 1984;**104**:172–6.

[22] Danne T, Grutes A, Schuppan D, Quantas N, Enders I, Webe B. Relationship of procollagen type III propeptide-related antigens in serum to somatic growth in healthy children and i patients with growth disorders. *J Pediatr* 1989;**114**:257–60.

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Laterality and prematurity

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SUMMARY Lateral preferences were determined by postal questionnaire for 240 children, without major neurological impairment, who were born at less than 31 weeks' gestation to examine the association between neonatal brain injuries and later lateral preference. Left hand preference was reported in 64 (26·7%) children at a median age of 52 months (range: 24–104 months). Left foot preference was shown by 70 (29·2%) and left eye preference by 86 (35·8%). Significantly more preterm children were left handed compared with parents or siblings in whom expected frequencies of left handedness were found. All index children had serial neonatal cerebral ultrasound examinations, of which 95 were abnormal. Left preference occurred at similar frequencies in those with normal neonatal scans (26·9%), bilateral lesions (28·3%), left sided lesions (23·8%), and right lesions (23·8%). Lateral preference appears to be unaffected by the side or extent of neonatal brain injury in children without major impairments.

Several studies have attempted to document an association between perinatal factors and the presence of left handedness using both specific markers, such as the time to establish spontaneous respiration after birth,[1] and more general indices such as optimality scores,[2] parity, and 'birth stress'.[3] In general, other studies have failed to confirm these findings.[4 5] There is, however, a high frequency of sinistrality among certain patient groups who have a high prevalence of neurological abnormality,[6] including those of very low birth weight.[7 8] Studies in children and adults with congenital and acquired hemiplegia show that major brain injury is important in the establishment of lateral preference.[9]

Among preterm children several causes for this observation have been postulated: a developmental defect,[7] asymmetric brain injury,[6] and the head turning preference of individual neonatal nurseries.[10 11] These studies were undertaken before cerebral imaging techniques were available. The high frequency with which abnormalities are observed on neonatal cerebral ultrasound scanning suggests that cortical brain injury may be an aetiological factor in the determination of hand preference in the very preterm population, and an observed excess of left sided ultrasound abnormality would seem to confirm this.[12]

The concept that biological handedness may be altered by organic brain injury (pathological hand preference) was introduced by Redlich in 1908.[8] Injury to the potential dominant hemisphere may result in transfer of function to the other. Central to this hypothesis is the presence within such populations of left and right handed individuals who have a pathological basis for their lateral preference. Organic brain injuries affect both biological left and right handed individuals, in proportion to their prevalence in the normal population. Assuming an equal distribution of predominantly left and right cortical insults, any brain injured population would show equal proportions of left and right handers. A knowledge of the frequency and laterality of brain lesions thus enables the prediction of the prevalence of left and right preference in a population.

Since 1980 routine cerebral ultrasound scans have been performed on all infants admitted to the neonatal unit at this hospital. This study documents the prevalence of lateral preference in children of less than 31 weeks' gestation in relation to family history and explores the hypothesis that lateral preference is related to the size and extent of neonatally observed brain lesions.

Subjects and methods

All high risk children managed on the Mersey Regional Neonatal Intensive Care Unit at Liverpool Maternity Hospital have been followed up prospectively since 1980. Children of less than 31 weeks' gestation were entered into the study if they were free of cerebral palsy or major visual impairment and over 2 years of age. A total of 302 parents of 331 children were circulated with a questionnaire asking for details of the hand, foot, and eye preference of the index child, parents, and siblings. Simple instructions were provided requesting that the parents observed their children for two days before completing the questionnaire, giving details of suitable tasks in which to observe each child—hand preference: writing, cutting, and using a spoon; foot preference: kicking a ball, lead foot when climbing stairs or walking on tiptoe; eye preference: 'telescope' of card or paper. Details of the gestation and birth weight of siblings was requested in order to provide a comparison group of term children. Preference was recorded as either left, right, or unsure for each item.

Since 1980 routine neonatal ultrasound scanning has been performed at least weekly during each infant's stay in the unit. Scans were photographed on Polaroid film and an independent assessment was undertaken by one observer (RWIC). Initially scans were performed with an ATL 850A scanner using 5 MHz transducers (1980–2) and latterly 7·5 MHz (1982 onwards). Lesions were classified by degree of abnormality and side. Periventricular haemorrhages were classified as subependymal, intraventricular, or intracerebral; late appearance as persistent ventricular enlargement, small periventricular cysts ($<$1·0 cm), large periventricular cysts ($>$1·0 cm), or shunted hydrocephalus.

Children were nursed in incubators arranged parallel to the nursery wall. Because of the normal excess of right preference among the nursing staff, children tended to be nursed with face to the right, which would tend to favour the development of right preference.

A standard deviation score of birth weight for gestational age for each child was calculated using the standards of Keen and Pearse.[13] This was analysed both as a dichotomous variable, using various cut off points, and as a continuous variable. Data were encoded and analysed using the Statistical Package for the Social Sciences (SPSS/PC+). The significance of differences in frequencies were tested using χ^2 statistics with Yates's correction, as appropriate.

Results

Replies were received for 240 (73%) index children, median age 56 months, range 24–104 months. Parental preference was recorded for 232 children. Altogether 90 children had 87 full term siblings over 2 years of age and a further 36 preterm siblings, 21 of whom were less than 31 weeks' gestation at birth. Median birth weight for respondents was 1100 g (range: 560–1500 g) and gestational age 28 weeks (range 24–30 weeks). Median SD score of birth weight for gestational age was −0·27 (range: −3·5 to +1·42). Only 10 children had a SD score below − 2·0 and 21 a score below −1·5.

Parents reported that 64 index children (26·7% showed left hand preference, 162 (67·5%) right preference, and 14 (5·8%) were unsure. Similar proportions for preference were reported for foot and eye 70 children (29·2%) showed left foot preference (1 (7·9%) unsure) and 86 (35·8%) left eye preferenc (24 (10%) unsure). Only 61 (25·4%) children were reported as showing consistent ipsilateral preference for hand, foot, and eye, while 154 (64·2%) showed preference for ipsilateral hand and eye. Simila proportions of right and left handed children showed preference for ipsilateral eye or foot Children with consistent ipsilateral preference were significantly more likely to show left hand preference (33 of 61 (54·1%)) than those without (31 o 179 (17·3%); χ^2 33·5; df=2; p$<$0·0001).

Of 87 full term siblings, 11 (12·6%) were reported to show left preference at a median age of 94 month (range 37–288 months). Index children with ful term siblings were significantly more likely to show left hand preference than their sibling (25 (27·8% χ^2 5·35; df=1, p=$<$0·05) and less likely to show ipsilateral preference for hand, foot and eye (siblings 75 (85·2%)) or hand and eye (siblings: 62 (70·5%)) The 87 full term siblings had similarly lower frequencies of left foot (nine (10·2%)) and left eye preference (17 (19·5%)) than index children. Only 3 index children had preterm siblings, of whom only four (11·1%) had left preference.

The frequency with which left hand preference was reported among parents and siblings varied from 7·8–14·4% (table 1) and was significantly lowe than that found among the index children. Lef handed fathers were significantly more common among preterm children with left preference whereas the frequency of left handed mothers and siblings did not vary significantly between groups (table 1). Stepwise multiple regression using the index child's hand preference as the dependent variable and family preferences as covariates failed to identify any further association. No effect of multiple pregnancy, gender, gestation, SD score of birth weight for gestation, or age at the time of testing could be identified.

One hundred and forty five children had norma cerebral ultrasound scans during the neonata period, 53 had bilateral abnormalities, and 4 lesions confined to the left or right side (table 2). O those with abnormal scans the frequency of left preference varied from 23·8–28·4% compared with 26·9% for those with normal scans. Breaking down the scan results by early appearance (periventricula

Table 1 *Association between hand preference of index children and family members. Results are No(%)*

	All index children		*Preference of index children*					
			Left		*Right*		*Unsure*	
Preference of family member:	*Left*	*Right*	*Left*	*Right*	*Left*	*Right*	*Left*	*Right*
Mother (n=232)	18 (7·5)	212 (88·3)	6 (9·4)	57 (89·1)	12 (7·6)	144 (91·7)	1 (8·3)	11 (91·7)
Father (n=232)	32 (13·8)	199 (85·8)	12 (18·8)	49 (76·6)	16 (10·2)	141 (89·8)	4 (30·8)	9 (69·2)
Siblings:								
Term (n=90)	13 (14·4)	71 (78·9)	5 (20·0)	19 (76·6)	8 (13·6)	47 (79·7)	0	5 (83·3)
Preterm (n=33)	4 (12·1)	28 (85·0)						

Table 2 *Association of hand preference in index children to neonatal ultrasound appearances. Results are No(%)*

	Total No	*Hand preference of index patients*		
		Right	*Left*	*Unsure*
No brain injury	145	98 (67·6)	39 (26·9)	8 (5·5)
Left sided lesions only:				
Any	21	16 (76·2)	5 (23·8)	
Haemorrhage (grade II+):	10	7 (70·0)	3 (30·0)	
Cystic changes (grade II+):	8	3 (37·5)	4 (50·0)	
Right sided lesions only:				
Any	21	16 (76·2)	5 (23·8)	
Haemorrhage (grade II+):	12	9 (75·0)	3 (25·0)	
Cystic changes (grade II+):	11	7 (63·6)	4 (36·4)	
Bilateral lesions:				
Any	53	32 (60·4)	15 (28·3)	6 (11·3)
Asymmetrical				
{ Left >right	9	5 (55·6)	3 (33·3)	1 (11·1)
{ Right >Left	8	6 (75·0)	2 (25·0)	

haemorrhage) or by late appearance (ventricular dilatation or cystic changes) showed a slight excess of left hand preference among the small number of children with unilateral left sided cystic changes. No other type or distribution of abnormalities appeared to affect hand preference or consistency of preference.

Discussion

Using a simple postal questionnaire we have demonstrated over twice the expected frequency of left preference among a population of very preterm children. This prevalence, based on a referral hospital population, is higher than a previous comparable report where 19% had left preference.[7] One might anticipate that there would be a bias towards the reporting of right preference among index children from the mainly right handed parents. This questionnaire is considerably less complex than those previously used in which some estimates of the certainty of handedness was included. The age at which parents were asked to assess their children was also younger than most studies in childhood. Although there is stability in preference after 5–7 years,[7] the median age of the index group was 56 months. The frequency with which parents reported left preference or uncertainty was similar in older and younger children.

For the normal population, the preference of left preference ranges from 9·2–17·5% within various reported study groups.[2 4 14–16] The preferences of the siblings and parents of the index children thus fall within the normal distribution. Among the family members males had a higher frequency of left preference as noted by other authors.[2] This difference is not apparent among very low birthweight children in this or one other study.[7] The frequencies of left eye and foot preference found in this study are similar to those observed in large population studies.[17 18] The proportion of index children who had mixed preference for hand and foot (22·6%) is consistent with studies of 7 year old children where 29·5% had mixed preference.[17]

Neonatal cerebral ultrasound scanning showed abnormalities in 95 (39·6%) children. Using the model proposed by Satz,[6] assuming a 50:50 distribution of lesions between left and right and a 10% prevalence of biological left preference, the predicted prevalence of left preference in the study population is 62 (25·8%) (figure). Sixty four children (26·7%) were reported as showing left hand preference. Despite this striking similarity, examination of the data taking account of the ultrasound abnormalities failed to confirm this association. The distribution of preference was similar in those with observed ultra-

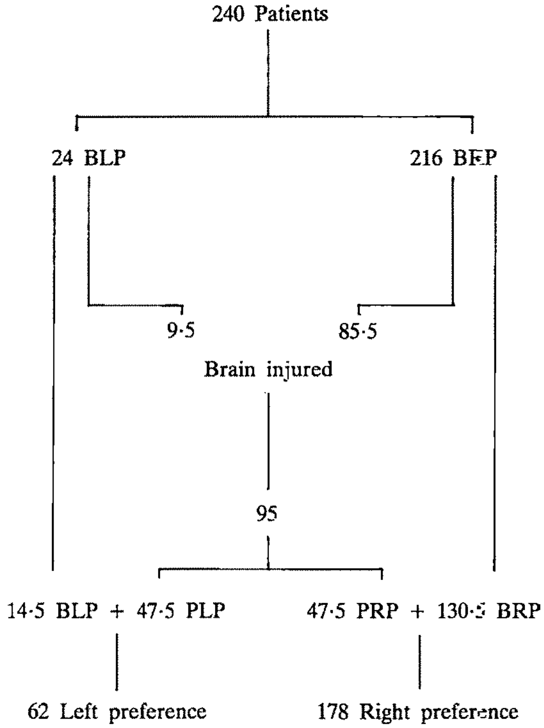

Figure *Theoretical distribution of hand preference based upon the model of Satz. Asumptions made are: (i) 10% population have biological left preference; (ii) there is a 39·6% brain injury rate; and (iii) there is a 50:50 distribution of side of greatest injury. (BL(R)P: biological left (right) preference; PL(R)P: pathological left (right) preference.)*

sound abnormality and those without, and similar in those with asymmetric lesions. When gross abnormalities detected by brain imaging do not clearly demonstrate the predicted effect on laterality it is difficult to hypothesise that less marked abnormalities may cause the observed excess of left preference.

Preterm children at school age have a range of abnormalities of motor, cognitive, behavioural, and neurological function. Data from Ross and colleagues suggest that these abnormalities are found more frequently among preterm children with non-right preference.[7] A subgroup of this index group has been assessed in detail during a study of motor skills in 53 children of <1251 g birth weight.[19] There was no relation between hand preference and motor performance in the index or control children (unpublished data). Rather it would appear that the altered distribution of preference parallels the abnormal neurological and cognitive maturation in preterm children as compared with term children. Further studies are in progress to chart the progress of two subgroups of this study population in terms of neuromotor and cognitive performance, including direct observation of lateral preference. The high frequency of left preference in this population remains unexplained.

References

[1] Barnes F. Temperament, adaptability and lefthanders. *New Scientist* 1975;**67**:202–3.

[2] Gillberg C, Waldenstrom E, Rasmussen P. Handedness in Swedish 10 year olds. *J Child Psychol Psychiatry* 1984;**25**: 421–32.

[3] Bakan P. Are lefthanders brain damaged? *New Scientist* 1975;**67**:200–2.

[4] Ounsted M, Cockburn J, Moar VA. Hand preference: its provenance, development and assocations with intellectual ability at the age of 7·5 years. *Developmental and Behavioural Pediatrics* 1985;**6**:76–80.

[5] McManus IC. Handedness and birth stress. *Psychol Med* 1981;**11**:485–96.

[6] Satz P. Pathological lefthandedness: an explanatory model. *Cortex* 1972;**8**:121–35.

[7] Ross G, Lipper EG, Auld PAM. Hand preference of four year old children: its relationship to premature birth and neurodevelopmental outcome. *Dev Med Child Neurol* 1987;**29**: 615–22.

[8] O'Callaghan MJ, Tudehope DI, Dugdale AE, Mohay H, Burns Y, Cook F. Handedness in children with birthweights below 1000 g. *Lancet* 1987;**i**:1155.

[9] Costeff H, Amikam R, Bergman M, *et al.* Eye sighting preference of normal and hemiplegic children and adults. *Dev Med Child Neurol* 1988;**30**:360–4.

[10] Michel GF, Harkins DA. Postural and lateral asymmetries in the ontogeny of handedness during infancy. *Dev Psychobiol* 1986;**19**:247–58.

[11] Konishi Y, Kuriyama M, Mikawa H, Suzuki J. The effect of body position on later postural and functional lateralities of preterm infants. *Dev Med Child Neurol* 1987;**29**:751–7.

[12] Monset-Couchard M, de Bethman O, Brouard-Orzechowski C, Relier JP. Grading periventricular haemorrhage in neonates with regard to laterality and parenchymal localisation. *J Radiol* 1987;**68**:159–66.

[13] Keen DV, Pearse RG. Weight, length, and head circumference curves for boys and girls of between 20 and 42 weeks' gestation. *Arch Dis Child* 1988;**63**:1170–1.

[14] Hardyck C, Petrinovich LF, Goldman RD. Lefthandedness and cognitive deficit. *Cortex* 1976;**12**:266–79.

[15] Bishop DVM. Handedness, clumsiness and cognitive ability. *Dev Med Child Neurol* 1980;**22**:569–79.

[16] Smart JL, Jeffrey C, Richards B. A retrospective study of the relationship between birth history and handedness at six years. *Early Hum Dev* 1980;**4**:79–88.

[17] Coren S, Porac C. Birth factors and laterality. *Behav Genet* 1980;**10**:123–8.

[18] Nachshon I, Denno D, Aurand S. Lateral preferences of hand, eye and foot: relation to cerebral dominance. *Int J Neurosci* 1983;**18**:1–10.

[19] Marlow N, Roberts BL, Cooke RWI. Motor skills in extremely low birthweight children at the age of 6 years. *Arch Dis Child* 1989;**64**:839–47.

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Fractional measurements of sweat osmolality in patients with cystic fibrosis

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SUMMARY After pilocarpine iontophoresis the change of sweat concentration during collection was studied by vapour pressure osmometry in 24 patients with cystic fibrosis and 24 healthy controls. There was a continuous but proportionate fall in sweat concentrations during the collection period. Mean (SD) initial sweat concentration in the control group was 154·4 (32·6) mmol/kg falling, after 50 µl of sweat produced, to 92·9 (15·8) mmol/kg. In the cystic fibrosis group it was 315·9 (35·8) mmol/kg falling to 247·4 (24·9) mmol/kg. Despite different rates of fall in concentrations, separation of the two groups was maintained throughout. We conclude that there are implications for the potential improvement of the predictive value of the sweat test.

The sweat test remains the most reliable laboratory procedure to confirm the clinical diagnosis of cystic fibrosis.[1 2] Despite attempts to develop new procedures[3–5] the method of sweat collection and analysis used in most centres is still based on that originally described by Gibson and Cooke.[6]

Reliable diagnosis by this method requires that a volume of sweat exceeding a predefined minimum is collected to avoid analytical error.[7] A recent study of the association between the sodium concentration and weight of sweat obtained by the Gibson and Cooke method in children with and without cystic fibrosis suggested that the predictive value of the test may be improved[8]; by plotting sweat weight against sodium concentration it was possible to separate the group with cystic fibrosis from the control group, when previously overlap had existed. Implicit from the analysis was that sweat sodium concentration is inversely related to the volume of sweat produced and that the concentration falls during the collection.

Developments in osmometry and sweat collection techniques have now made the direct measurement of sweat osmolality possible.[9–11] The capillary sweat collection system (Macroduct), carried out as recommended by the manufacturers, collects sweat in fractions, but pools or cumulates the completed collection for analysis.

Using the Macroduct system the present study was carried out to determine if more diagnostic information could be obtained from fractional sweat concentrations than from the conventional procedure using pooled samples.

Subjects and methods

Twenty four patients who fulfilled the clinical criteria for cystic fibrosis and who had all previously been shown to have abnormal sweat test results by the method of Gibson and Cooke (minimum sweat weight 100 mg, sodium concentration >60 mmol/l, chloride concentration >60 mmol/l) were matched with 24 healthy control subjects. There were 11 boys and 13 girls with a mean age of 10·0 years (range 5–15 years) with cystic fibrosis, and the normal control group comprised 15 boys and nine girls with a mean age of 8·3 years (range 1–13 years). Ethical approval was granted by the South Gwent ethical committee.

Sweat collection was carried out using a modified Wescor Macroduct system (Chem Lab Instruments). The modification allowed the reuse of concave collector discs by replacing the central capillary tube with polythene tubing 0·8 mm diameter×120 mm (Hilger Analytical). Sweating was stimulated using 25 mm diameter×5 mm 2% agarose in water discs containing 0·5% pilocarpine, and iontophoresis was induced for five minutes. After thorough cleaning of the skin, sweat was subsequently collected from the stimulation site for 30 minutes. The capillary tube was then gently clamped at both proximal (to the skin) and distal ends of the fluid using fine artery forceps and carefully removed from the collector disc. The tubing was placed on a level surface and the clamps released. Unfilled distal tubing was trimmed using a scalpel, and sweat was drawn from this end in 10 μl aliquots with an automatic pipette. To minimise diffusion effects all measurements of osmolality were carried out within 30 minutes of collection using a precalibrated Wescor Vapour Pressure Osmometer 5500.

Statistical analysis was by unpaired Student's *t* test, cumulative frequency analysis, and linear regression analysis.

Results

The individual values and amount of sweat collected (to the nearest complete 10 μl) are shown in fig 1.

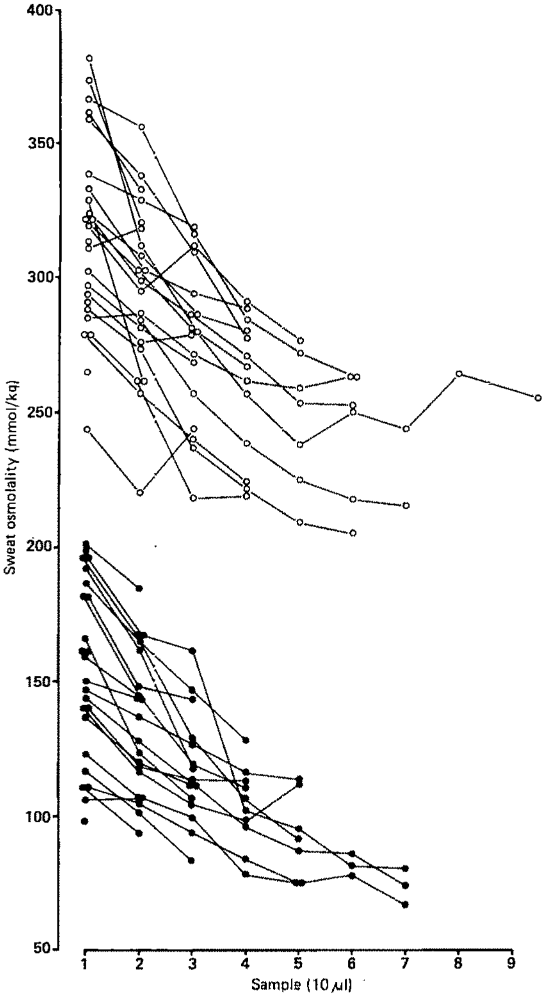

Fig 1 *Fractional sweat osmolality measurements in normal children (●) and children with cystic fibrosis (○).*

There was a consistent and significant fall in osmolality throughout the collection in both groups. As only the initial 10 μl of sweat collected is common to all patients and controls the cumulative frequency distributions of the initial data on sweat osmolality are plotted in fig 2. The data are normally distributed within both groups with complete and parallel separation.

The influence of sweat production rate (as measured by 30 minute volume) on the osmolality of the first aliquot of sweat was studied by linear regression analysis. No significant correlation in either group was established (cystic fibrosis group $r=0{\cdot}04$, $p>0{\cdot}05$; control group $r=0{\cdot}08$, $p>0{\cdot}05$).

It is possible to calculate the sweat osmolality

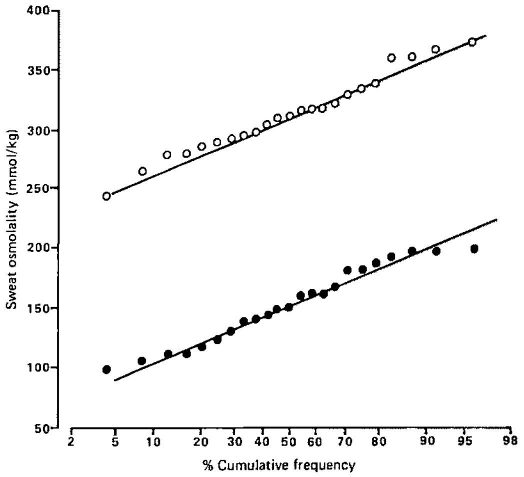

Fig 2 *Cumulative frequency distribution of the osmolality measurement in the initial 10 μl of sweat collected from normal children (●) and children with cystic fibrosis (○).*

Table *Sweat rates and osmolalities of both pooled sweat and the initial 10 μl collected from 24 normal children and 24 children with cystic fibrosis. Values are expressed as mean (SD)*

	Normal children	*Children with cystic fibrosis*	*t*	*p Value*
Sweating rate (ml/min)	1·14 (0·64)	1·25 (0·67)	0·58	>0·5
Osmolality of pooled sweat	134·2 (31·4)	289·4 (31·7)	17·0	<0·001
Range	88–199	236–348		
Osmolality of initial sample	154·4 (32·6)	315·9 (35·8)	16·3	<0·001
Range	98–202	244–382		

result that would have been obtained in an individual subject by the conventional procedure of pooling sweat before analysis—this is simply achieved by calculating the mean result of all the fractions. As observed, there is potential for overlap of the groups if the volume of sweat is not taken into account (that is, comparison of a patient with cystic fibrosis who produced a large volume of sweat and a normal control subject who produced only a small volume). The difference between the range of sweat osmolality values obtained from the group with cystic fibrosis and that from the normal control group is highly significantly different on comparison of the calculated pooled osmolality values (table). The significance is not further enhanced by examination of the initial 10 μl sweat volume fraction, but by analysis of the subsequent sweat concentrations better separation and improved significance can be achieved. Fig 3 shows the mean and 95% confidence intervals of the individual fraction values dependent on volume of sweat produced for the first five fractions. With each successive aliquot of sweat produced the two groups separate. The sweat concentration in either group reduces in a proportional or first order manner. The rate of reduction of log mean sweat osmolality with sweat volume is greater in the control group than in the group with cystic fibrosis (fig 4), hence the improved separation is achieved.

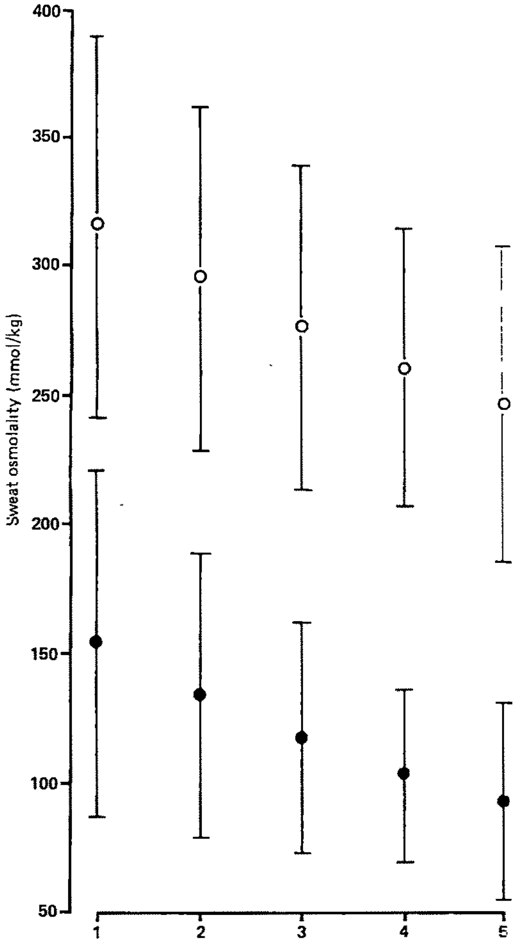

Fig 3 *Mean and 95% confidence intervals of the sweat osmolality measurements for each of the first five fractions of sweat collected in normal children (●) and children with cystic fibrosis (○).*

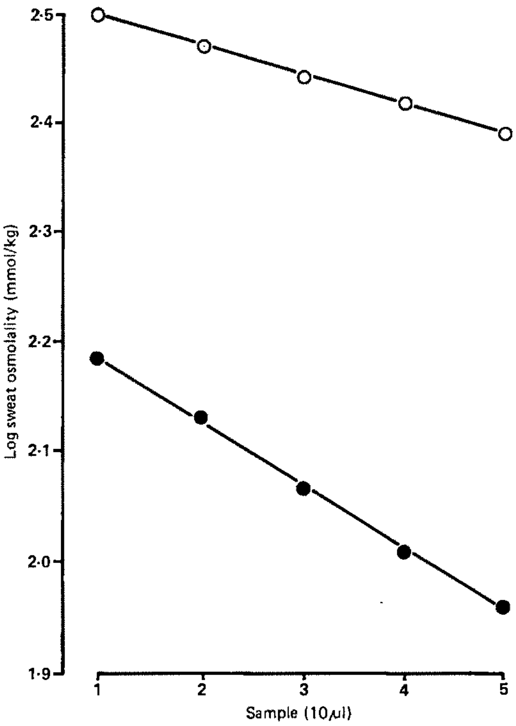

Fig 4 *Rate of reduction of mean sweat osmolality (log scale) and sweat volume collected in normal children (●) and children with cystic fibrosis (○).*

Discussion

There is increasing evidence that measurement of sweat osmolality is a reliable method for the diagnosis of cystic fibrosis. Our results of pooled individual data would concur with the range of diagnostic values established by others.[9-11] In the two groups studied we have shown that there is clear diagnostic separation. This parallel separation of cumulative frequency plots of initial sweat osmolality is, as far as we are aware, the first time that the influence of volume on the concentration achieved has been excluded.

The production of sweat in fractions has permitted the study of changes in sweat concentration with volume. There is a dramatic reduction in sweat concentration as the volume of sweat that is produced increases, and thus the origin of potential overlap of groups is apparent. In practice, however, the use of the only consistent fraction between subjects—that is the first aliquot of sweat—as a discriminant of groups would not seem to offer an advantage over pooled sweat collected in the conventional manner. The reason for this is the fortuitous advantage of studying a pool rather than just one aliquot, accentuating the differing proportional rates of sweat osmolality reduction in the two groups. It should be emphasised, however, that separation has been achieved at all volumes of sweat. There is no obvious minimum sweat volume necessary for diagnostic discrimination and if this volume exists, it is currently dependent only on the restrictions of instrumentation.

How much longer the sweat test will remain as the basic diagnostic laboratory investigation for cystic fibrosis is open to conjecture now that molecular biology techniques are being developed.[12-14] Irrespective of its long term role in this regard, however, we believe the changes in sweat concentration may have important implications for the application of sweat solute analysis for other metabolic disorders and in the overall understanding of the physiology of sweat production. For the near future the sweat test will continue as the laboratory investigation of choice to confirm the diagnosis.

In this study we have confirmed that measurement of sweat osmolality is a reliable analytical method. The need for only a small quantity of sweat means that problems in obtaining sweat in children who are poor producers of sweat, or in neonates, may be overcome. Moreover, we have shown that diagnostic difficulties resulting from borderline results may be overcome by a fractional technique and the application of appropriate reference intervals, particularly if high overall sweat volumes can be achieved.

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References

[1] Division of Medical Sciences, Assembly of Life Sciences, National Research Council, National Academy of Sciences, Washington DC. Report of the Committee for a Study for Evaluation of Testing for Cystic Fibrosis. *J Pediatr* 1975;**88**:711–50.

[2] Littlewood JM. The sweat test. *Arch Dis Child* 1986;**61**:1041–3.

[3] Shwachman H, Dunham R, Phillips WR. Electrical conductivity of sweat. A simple diagnostic test in children. *Pediatrics* 1963;**32**:85–8.

[4] Warwick WJ, Hansen L. The silver electrode method for rapid analysis of sweat chloride. *Pediatrics* 1965;**36**:261–4.

[5] Mei V, Commellini L, Secciani L, Paulucci G. Total osmolality and principal osmoactive constituents of sweat in subjects with mucoviscidosis and their families. *Boll Soc Ital Biol Sper* 1966;**42**:1388–90.

[6] Gibson LE, Cooke RE. A test for concentration of electrolytes in sweat in cystic fibrosis of the pancreas utilizing pilocarpine by iontophoresis. *Pediatrics* 1959;**23**:545–9.

[7] Gibson LE. The decline of the sweat test. *Clin Pediatr* 1973;**12**:450–3.

[8] Hjelm M, Brown P, Bridden A. Sweat sodium related to amount of sweat after sweat test in children with and without cystic fibrosis. *Acta Paediatr Scand* 1986;**75**:652–6.

[9] Webster HL, Barlow WK. New approach to cystic fibrosis diagnosis by use of an improved sweat induction/collection system and osmometry. *Clin Chem* 1981;**27**:385–7.

[10] Kirk JM, Adams A, Westwood A, McCrae WM. Measurement of osmolality and sodium concentration in heated-cup sweat collections for the investigation of cystic fibrosis. *Ann Clin Biochem* 1983;**20**:369–73.

[11] Carter EP, Barrett AD, Heeley AF, Kuzemko JA. Improved sweat test method for the diagnosis of cystic fibrosis. *Arch Dis Child* 1984;**59**:919–22.

[12] Knowlton RC, Haguenauss O, Buchwald M, *et al.* A polymorphic DNA marker linked to cystic fibrosis is located on chromosome 7. *Nature* 1985;**318**:380–2.

[13] White R, Woodward S, Dear M. A closely linked genetic marker for cystic fibrosis. *Nature* 1985;**318**:382–4.

[14] Wainwright B, Scambler P, Williamson R. Localisation of cystic fibrosis locus to human chromosome 7. *Nature* 1985;**318**:384–5.

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Adrenal response in very low birthweight babies after dexamethasone treatment for bronchopulmonary dysplasia

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SUMMARY The tetracosactrin stimulation test was used to assess the adrenal responsiveness of 22 very low birthweight babies who had received a three week course of dexamethasone for the treatment of bronchopulmonary dysplasia. Five babies were studied in detail with blood samples taken for cortisol concentrations at 30 minute intervals for four hours. The tests were performed before, during, and after treatment with dexamethasone. A distinctive pattern of cortisol response to tetracosactrin was found among these babies, which was quite unlike that found in older children and adults. Using our pretreatment results as control data we conclude that there is undoubtedly evidence of modest suppression of the adrenal axis during dexamethosone treatment, although there is considerable recovery one month after stopping steroids. Basal cortisol concentrations, however, remained low in some cases, which may indicate the need for temporary corticosteroid replacement during severe illness.

Corticosteroids are being used increasingly for the treatment of bronchopulmonary dysplasia in preterm babies. If the British multicentre trial now underway reports favourably on the effectiveness of steroids in this condition, their use will undoubtedly increase further. We have already observed that a course of dexamethasone, in the dose of 0·6 mg/kg/day, can bring about a dramatic improvement in lung function and subsequent reduction in ventilatory requirements in many babies with bronchopulmonary dysplasia. Some cases, however, seem to relapse at the end of the one week course of dexamethasone usually prescribed. In an attempt to prevent relapse we have extended the course to three weeks, tapering the dose progressively after the first week. As the literature contains little, if any, information on the effect of steroids on the adrenal gland of very low birthweight babies, we have undertaken to perform a series of tetracosactrin stimulation tests on 22 babies (five studied in detail) whom we have treated with steroids during the past 18 months.

Patients and methods

The investigation was performed on 22 very low birthweight babies admitted to the regional neonatal unit at St James's University Hospital, Leeds. None of their mothers had received steroids during pregnancy. All the babies required ventilation from birth for respiratory distress syndrome and had developed bronchopulmonary dysplasia of sufficient severity to prevent them from coming off ventilatory support. Each baby was given a full three week course of dexamethasone: 0·6 mg/kg/day for the first week, half of this dose in the second week, and a quarter of it in the third week.

Tetracosactrin stimulation tests were performed on day 0, just before dexamethasone was started, at the end of week 1 and at the end of the three week course. If babies were still in the unit one month after steroid treatment finished (that is, week 7), a further test was performed. All tests were performed at 9 am. Adrenal stimulation was produced by intramuscular injection of 125 μg of tetracosactrin (Synacthen, Ciba). In 17 babies, a standard test was performed, with blood samples taken immediately before, and 30 minutes after, tetracosactrin. Almost all samples were obtained from indwelling arterial lines but if this access was unavailable when a sample was needed, capillary blood was used.

In five babies, who had indwelling arterial lines on each occasion a test was due, we performed a prolonged test, with samples taken at 30 minute intervals for four hours.

Fully informed parental consent was obtained in each case. The study was approved by the hospital clinical research (ethics) committee.

Plasma was extracted and stored at 4°C. All samples were analysed within 48 hours. Plasma

Table 1 *Clinical data and plasma cortisol concentration at 0 and 30 minutes in babies studied with conventional tetracosactrin test (n=17)*

Case No	*Gestation (weeks)*	*Weight (g)*	*Age steroid started (days)*	*Cortisol concentration (nmol/l)*							
				Day 0		*Week 1*		*Week 3*		*Week 7*	
				0 Min	*30 Min*	*0 Min*	*30 Min*	*0 Min*	*30 Min*	*0 Min*	*30 Min*
1	27	750	21	378	899	59	571	60	630	175	852
2	25	630	21	148	923	74	612	163	886	2	477
3	30	1150	13	150	501	136	413	110	471	68	341
4	26	895	70	85	786	15	246	18	640	13	802
5	30	1236	28	55	398	18	128	53	270	76	601
6	30	955	28	188	373	69	281	59	331	—	—
7	26	875	32	309	551	127	512	53	531	—	—
8	30	1300	16	183	771	152	590	37	347	125	721
9	27	1120	10	204	746	162	622	312	623	—	—
10	26	815	35	198	445	58	324	37	238	—	—
11	26	979	25	209	768	110	620	249	830	—	—
12	26	849	14	242	1048	57	846	67	626	83	675
13	27	673	21	272	482	84	528	86	490	—	—
14	32	1500	34	455	850	40	203	12	603	—	—
15	28	1378	16	125	705	57	548	75	865	92	837
16	28	1070	46	138	577	104	325	—	—	—	—
17	25	835	17	217	674	163	782	—	—	—	—

Table 2 *Clinical data and cortisol concentrations in the five babies studied with an extended tetracosactrin test*

Case No	*Gestation (weeks)*	*Weight (g)*	*Age steroid started (days)*	*Stage*	*Cortisol concentration (nmol/l)*								
					0 (min)	*30*	*60*	*90*	*120*	*150*	*180*	*210*	*240*
18	26	1202	19	Day 0	580	920	1240	1480	1830	—	—	—	—
				Week 1	411	552	723	945	1044	1230	1392	—	—
				Week 3	35	419	580	641	782	832	769	592	518
				Week 7	16	479	840	1147	1193	954	729	495	334
19	26	976	23	Day 0	399	848	1290	1254	1620	1926	1956	2220	2322
				Week 1	37	476	867	1054	1217	1117	1276	1218	1153
				Week 3	47	356	582	786	847	861	978	912	821
				Week 7	98	397	577	880	923	869	632	527	420
20	26	1120	19	Day 0	275	545	658	767	914	1087	1145	1090	1276
				Week 1	79	451	593	685	728	902	1033	1090	1249
				Week 3	49	328	438	489	516	620	631	652	597
				Week 7	74	437	718	1011	1316	1105	806	544	823
21	29	860	16	Day 0	280	755	941	1199	1178	1338	1411	1793	1684
				Week 1	38	408	534	645	721	738	832	808	728
				Week 3	26	327	555	586	769	797	735	884	802
				Week 7	77	857	1130	1400	1510	1376	1237	934	815
22	27	1059	13	Day 0	172	844	1338	1321	1616	1874	2040	2172	2240
				Week 1	38	557	808	970	1057	1180	1314	—	1361
				Week 3	135	777	934	1105	1381	1414	1542	1562	1506
				Week 7	626	1276	2142	2364	2304	2343	2571	2247	2184

cortisol was measured in duplicate using a fluorescence polarisation immunoassay technique with the Abbott diagnostic TDx analyser. Cross reactivity with endogenous steroids was: corticosterone 4·5% and cortisone 1·1%, and with synthetic steroids: prednisolone 23·8%, prednisone 0·4%, and dexamethasone 0·6%.

Paired *t* tests and simple regression were used for statistical analysis where appropriate.

Results

The clinical data and the results of the tetracosactrin test at various stages are summarised in tables 1 and 2. Table 1 concerns the 17 babies who had a standard test and table 2 those who had the extended test. Data is missing as follows: one patient in the extended study group did not have his cortisol concentration monitored for the full four hours as we were not initially aware of the prolonged nature of the response; two patients returned to their referring hospitals before the course of dexamethasone was completed and so missed both the week 3 and the final tetracosactrin test, and six others went home before the last test was due; two patients died, one before the final test was performed.

Fig 1 shows the mean plasma cortisol responses of the five babies studied in detail, at the four different stages in relation to dexamethasone treatment. On day 0, the cortisol concentration rose steeply and was still rising at four hours. The cortisol responses on weeks 1 and 3 were diminished in magnitude in proportion to the duration of dexamethasone treatment and the curves showed signs of becoming asymptotic after about 120 minutes. One month after stopping steroid treatment the cortisol responses consistently peaked at 120 minutes and then declined rapidly.

These different patterns of response make it difficult to fix on an ideal time after tetracosactrin stimulation at which to measure the cortisol concentration. Because we felt that the concentration at 120 minutes was probably the most informative, we were interested to see how the cortisol at this time correlated with the conventional 30 minute and 60 minute concentrations. Simple regression analysis was used to examine the correlation. Both the 30 minute ($n=20$) (fig 2) and 60 minute ($n=20$) cortisol concentrations, at the four stages of treatment, correlated well with the corresponding 120 minute concentration ($n=20$); the correlation coefficients (r values) were 0·94 and 0·95 respectively ($p<0{\cdot}00001$). The correlation between 30 and 60 minutes was also excellent ($r=0{\cdot}96$, $p<0{\cdot}00001$).

When the cortisol concentrations before dexamethasone treatment (day 0) were compared with later concentrations in the same baby, we found that after one and three weeks of dexamethasone the cortisol concentration at all sampling times, up to 180 minutes, was significantly lower than before dexamethasone treatment was started ($p<0{\cdot}035$, paired *t* test). In general, the results in the week 3 test showed a greater suppression than in week 1. By contrast, all week 7 results were not significantly different from control ($p>0{\cdot}08$, paired *t* test).

Paired *t* tests were also used to compare the increment in cortisol concentration (that is, the increase above prestimulation concentration) between the test before dexamethasone and later tests. Significance differences were found on very few occasions—at 90, 120, 150, 180, 210, and 240 minutes in week 3 ($p<0{\cdot}03$); at 30 minutes in week 1 ($p<0{\cdot}05$), and at 90 minutes in week 7 ($p<0{\cdot}05$).

When the basal cortisol values of all babies were pooled and compared at various times in relation to

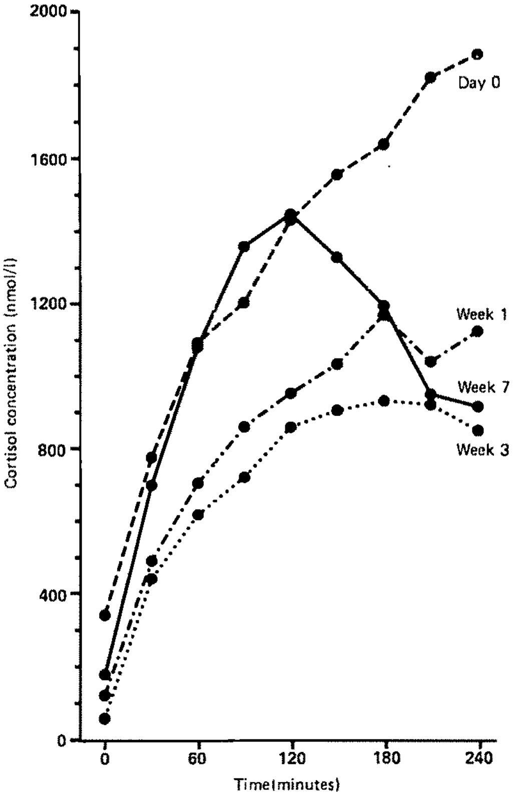

Fig 1 *Mean plasma cortisol concentrations of five babies studied by the extended tetracosactrin test at four stages in relation to dexamethasone treatment.*

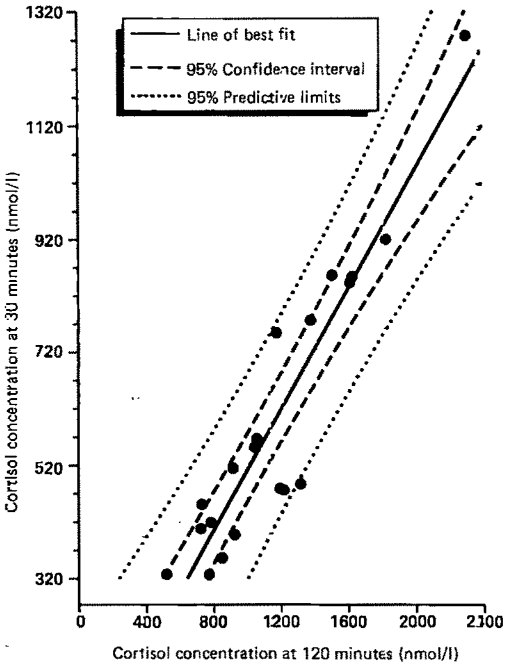

Fig 2 *Correlation between cortisol concentrations at 30 and 120 minutes.*

dexamethasone treatment, they were seen to fall from a mean of 239 nmol/l before treatment to 95 and 84 nmol/l, after one and three weeks of dexamethasone respectively ($p<0{\cdot}001$). There was a difference between the mean pretreatment basal concentration of 239 nmol/l and the mean basal concentration of 117 nmol/l one month after the end of treatment, but this was not significant ($p=0{\cdot}08$).

The correlation between the dose of tetracosactrin, expressed per kg body weight, and both the 30 minute and incremental cortisol response before dexamethasone treatment was examined. Correlation coefficients of 0·06 and 0·11 respectively were obtained ($p=0{\cdot}78$ and 0·63).

Correlations were also performed between 30 minute cortisol concentration and both gestational age and postconceptional age. The coefficients were −0·29 and −0·32 respectively ($p=0{\cdot}20$ and 0·16). The correlation coefficients between the incremental response, and gestational and postconceptional age were −0·26 and −0·25 respectively ($p=0{\cdot}27$ and 0·29).

Discussion

Suppression of adrenal gland responsiveness is one of the most serious side effects of steroid treatment, and a case of adrenal insufficiency has been reported in a neonate whose mother had received long term steroid treatment during pregnancy.[1] As dexamethasone is now being prescribed for the treatment of bronchopulmonary dysplasia, an understanding of its effect on adrenal responsiveness in very low birthweight babies is important. A literature search shows that information on adrenal function in this group of patients is scanty and at times confusing. The main source of confusion is the variable methodology used for performing tetracosactrin stimulation tests, both in terms of the size of the tetracosactrin dose and the timing of the blood sample taken after stimulation.[2-9] As a result there is in our opinion no reliable reference range against which to assess the adequacy of the adrenal response of very low birthweight babies. We chose to standardise on 125 µg of tetracosactrin, which is a large weight related dose for our smallest babies, in order to produce maximum stimulation of the adrenal gland. This was to avoid overdiagnosing adrenal suppression, which we felt was a risk in view of the paucity of knowledge about the adrenal response in such immature babies as were included in our study. In fact, our results showed no significant correlation between the dose of tetracosactrin/kg body weight and either the 30 minute cortisol concentration or increment.

We have sought to overcome some of the difficulties of interpretation of the test by studying a small number of babies in detail, so as to delineate the cortisol response more thoroughly. Also by performing tetracosactrin tests immediately before starting dexamethasone, we aimed to provide a reference point against which later responses might be judged.

In the extended study group we observed a progressive change in the shape of the response curves (fig 1), and several explanations can be offered. One possibility is that there was no effect of dexamethasone and we simply observed the normal maturational changes of the adrenal gland during late fetal and early neonatal life. To examine this in more detail we looked for a correlation between adrenal response and both gestational and postconceptional age before dexamethasone treatment. The fact that there was no significant correlation between either gestational or postconceptional age and any aspect of cortisol response suggests that maturational changes during this period are not the explanation for the observed changes in response. Another explanation, which is more plausible and could be contributory, is that the change in response might be stress related, as many of the babies were becoming progressively less ill as time went by. In this respect it is interesting to note that the sickest baby in the study (table 2, case 22), who did not respond to dexamethasone and who died of bronchopulmonary dysplasia, had the greatest cortisol response to tetracosactrin at weeks 3 and 7. The most important factor in our view, however, is the effect of dexamethasone on the hypothalamic-pituitary-adrenal axis, with partial recovery after a month without steroids.

Although in most reports either the 30 or 60 minute concentrations are considered suitable end points for the tetracosactrin test,[2-5 7-9] we suspect from our data that they do not represent the most appropriate times to use in this age group. Results from our extended study indicate that concentrations at these times do not represent peak concentrations. We suggest that the 120 minute concentration might represent the best compromise in this group of patients. This is because at week 7 the cortisol concentration consistently peaked at this time, and at the earlier stages (that is, weeks 1 and 3) it was very close to its asymptote. At week 0, although far from the peak, the 120 minute concentration at least allows time for a substantial rise in plasma cortisol to occur. It was reassuring to us, and should be to other investigators, to find that the often used 30 and 60 minute concentrations correlate very well with that found at 120 minutes (fig 2).

The fall in basal cortisol concentration after one and three weeks of dexamethasone was most likely due to suppression of the hypothalamic-pituitary higher centres by dexamethasone. The mean basal concentration four weeks after stopping dexamethasone was also lower than it was before starting dexamethasone, although not significantly so. As there was no lessening of the response after tetracosactrin stimulation at week 7, this observation raises the possibility of a more severe and prolonged effect of steroid treatment on higher centres.

Tetracosactrin stimulation tests are conventionally evaluated by comparison with reference ranges for representative populations. Commonly advocated criteria for a satisfactory response in children are an increment of >200 nmol/l or a 30 minute value of >500 nmol/l.[10] By the first of these criteria one of our 22 patients would be judged under-responsive before dexamethasone, three of them after one week's treatment, and none after three and seven weeks. By the standard of a 30 minutes concentration >500 nmol/l, four out of 22 would be judged under-responsive before dexamethasone, 10 out of 22 after one week's treatment, 10 out of 20 after three weeks' treatment, and five out of 13 at week 7. As it is unlikely, from our extended data, that any of our babies had reached their peak cortisol concentrations at the time of the 30 minute sampling, our data probably overestimate the incidence of under-responsiveness as judged by the criterion of the 30 minute peak concentration. In fact the data from the extended study group show that none of these five cases had a response which failed to fulfil either of the above criteria at any stage.

When comparisons of incremental response were made using the pretreatment concentration (day 0) as control, the response was significantly reduced only at week 3. By contrast, the absolute cortisol concentrations in week 1 and 3 were significantly lower than on day 0 at all times after stimulation. At week 7, neither the increments nor the absolute concentrations were significantly different from those before treatment. Interpretation of results is undoubtedly complicated by the lack of universal agreement on which of the three pieces of information obtained from the tetracosactrin test—the basal cortisol, the peak cortisol, or the increment—is the best measure of adrenocortical responsiveness.[11] One of the problems is the extent to which the three measures are interdependent. It seems clear, however, that in our babies some degree of adrenal suppression had taken place after one week of treatment and appeared to be most severe at the end of the three week course. One month after stopping steroids the adrenal gland was probably responding within normal limits. Throughout the treatment and recovery period none of the babies showed any signs of clinical or biochemical adrenal insufficiency.

We conclude that a course of dexamethasone as described in this study is relatively safe in terms of adrenal suppression and, at first glance, it would seem unnecessary to give steroid replacement at times of stress. The low basal cortisol concentrations found in some patients four weeks after stopping steroids, however, suggest that there may still be a degree of suppression of the hypothalamic and pituitary higher centres, which could indicate the need for steroid or adrenocorticotrophic hormone treatment during severe illness.

References

[1] Grajwer LA, Lilien LA, Pildes RA. Neonatal subclinical adrenal insufficiency. Result of maternal steroid therapy. *JAMA* 1977;**238**:1279–80.

[2] Noguchi A, Reynolds JW. Serum cortisol and dehydroepiandrosterone sulfate responses to adrenocorticotropin stimulation in premature infants. *Pediatr Res* 1978;**12**:1057–61.

[3] Barnhart BJ, Carlson CV, Reynolds JW. Adrenal cortical function in the postmature fetus and newborn infant. *Pediatr Res* 1980;**14**:1367–9.

[4] Arad I, Landau H. Adrenocortical reserve of neonates born of long-term, steroid-treated mothers. *Eur J Pediatr* 1984;**142**:279–80.

[5] Thomas S, Murphy JF, Dyas J, Ryalls M, Hughes IA. Response to ACTH in the newborn. *Arch Dis Child* 1986;**61**:57–60.

[6] Gutai J, George R, Koeff S, Bacon GE. Adrenal response to

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Psychiatric disorders in general paediatric referrals

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SUMMARY Detailed interviews with parents of 128 children aged 7 to 12 years consecutively referred to general paediatric clinics identified psychiatric disturbance in 36 (28%) of the children. Emotional disorders were the commonest psychiatric diagnoses (present in two thirds); less frequent diagnoses were conduct disorders (5/36, 14%), mixed conduct/emotional disorders (six, 17%), and hyperkinetic syndrome (three, 8%). Disturbance was related to level of energy, with disturbed children being described significantly more frequently as 'bounding with energy' or conversely 'tired and apathetic'. Psychosocial problems (broken homes, mentally distressed mothers, family stress including financial stress and marital difficulty) were also increased in the disturbed group, but most of all these mothers reported feeling stressed in relation to the children.

Psychiatric disorders, particularly emotional disorders, are common associated problems in paediatric referrals. Family stress, specially that focused on parenting, is likely to be an important factor contributing to disturbed children's consultations in general paediatric clinics.

Child mental health problems are a common reason for consultation in general paediatric clinics. They have been reported as the main reason for attending in between 5 to 10% of children in several surveys.[1-5]

There are indications that childhood psychiatric morbidity may also be present as an associated or background factor in children with somatic presentations,[6] but recognition of these behavioural problems by physicians is probably limited.[7] In contrast with the work done in adult medical patients, which has documented psychiatric morbidity in between a quarter and 40% of medical patients and physician recognition rates of between 40–50%,[8] these issues have received little direct research attention in the paediatric literature.

More information on the frequency, nature, and associations of childhood psychiatric morbidity among paediatric patients is clearly necessary if these problems are to be recognised and attended to, if appropriate, in paediatric clinics.

Two studies have used child behavioural questionnaires completed by parents in children attending general paediatric clinics and reported high rates of psychological deviance in over 35% of children.[9 10] The use of these questionnaires has, however, important limitations as they cannot ascertain whether the identified psychological symptoms reflect the existence of psychiatric syndromes that are appreciable and handicapping to the child as opposed to more trivial difficulties. The results therefore need replicating with more robust methodology assessing child psychiatric disturbance with valid research interviews.

We used such a procedure to study attendances to general practitioners of children 7 to 12 years of age. Mental health problems were the main reason for the presentations in 2% but psychiatric disturbance was present in 23% of children in most cases as a background or associated factor to somatic presentations.[11] Our findings indicated that disturbed children consulted more than other children and disturbance tended to be linked to a variety of presentations such as hayfever, bedwetting, nosebleeds, and scabies. They also suggested that family stress, and a particularly pronounced parental concern about disturbed children and their behaviour, might have sensitised these parents towards their children's physical symptoms and contributed to their increased consultations.

We have extended this enquiry to examine the contribution of psychiatric problems at the paediatric outpatient clinic. In this paper we present data obtained from detailed psychiatric interviews with the parents to assess: (i) the frequency and nature of childhood psychiatric disorder in this setting and (ii) the associations of disturbance with somatic symptomatology, as well as with psychosocial family difficulty.

Subjects and methods

Consecutive new referrals of children aged 7 to 12 years to general paediatric clinics were selected for study. The children attended clinics run during 1986–7 by six consultant paediatricians with clinical responsibilities in four health districts covering a mixed population in Greater Manchester and Cheshire. Two districts were in city urban or suburban areas and the other two covered medium sized industrial towns and the surrounding rural areas. Only routine referrals entered the study; casualty referrals and those attending special interest clinics were not included and, to avoid language difficulties in completing questionnaires and interviews, children of immigrant families were also excluded.

Parents (mostly mothers) of eligible children were approached at the time of their first visit to the paediatric clinic. They were asked to complete questionnaires and their permission obtained to be interviewed at home. The questionnaires and interview inquired about demographic factors, presenting complaints, other somatic symptoms, recent medical contacts, and developmental problems in the child (modified child health questionnaire[12]), about child psychiatric symptoms, and about family function and stress.

ASSESSMENT OF PSYCHIATRIC DISORDER IN THE CHILD

To assess psychiatric disorder a clinicodiagnostic approach was used whereby detailed information about childhood psychiatric symptomatology was obtained systematically from the parents and a clinical diagnosis was made by a child psychiatrist (MEG) on the basis of this information. The recently revised version of the psychiatric interview with the parents was used.[12] This instrument has been used extensively in previous epidemiological studies of childhood psychiatric disorder, it has good inter-rater reliability and it has been found to be the best single method to assess disorder in children.[11 12]

For this interview, the parents are asked whether they feel the child has emotional or behavioural difficulties or nervous trouble and then systematically about the main clinically relevant areas (behaviour, emotions and social relationships, somatic symptomatology). Enough information is obtained to assess not simply whether the symptoms are present, but whether they are severe and handicapping. The diagnosis of psychiatric disorder requires the presence of an abnormality of behaviour, emotions, or relationships continuing up to the time of assessment and sufficiently pronounced or prolonged to cause handicap to the child himself and distress or disturbance in the family or community.[12] It excludes minor or trivial behavioural problems, whic may not deserve specialist attention and also pur 'psychosomatic' or learning problems and ment retardation. In this study, if the child had a disorde it was classified according to the main symptomato ogy into emotional, conduct, mixed conduc emotional disorders, hyperkinetic syndrome, or an other appropriate child psychiatric condition according to the International Classification of Di ease, 9th edition (ICD9).[13]

The reliability of the research psychiatrist's asses ments from the psychiatric interview with paren was tested in our previous study of psychiatric di order among children attending general practice. Twenty schedules were scored independently an blindly as to psychiatric state by another chil psychiatrist. The overall agreement for the presenc or absence of disorder was 80%, a similar rate t previous reports,[12] with an acceptable $\varkappa$ coefficie of 0·6.

As previous studies have employed questionnai information to assess psychological problems in chi dren, we used the child behavioural parental scale and teacher scale B for comparison purposes wit these studies and to supplement the parental wit school based information.[12] These scales consist of list of symptoms or items whose relative frequen and severity is rated on a three point scale if it h occurred in the previous year. A total score is com puted: scores of 13 or more (parental scale) and of or more (teacher scale) are regarded as indicatin psychological problems in the child and high risk f psychiatric disorder.

ASSESSMENT OF FAMILY STRESS AND DYSFUNCTION

We assessed parental mental health using the gene ral health questionnaire,[14] and we enquired abou other family health problems. To assess contempo ary psychological stresses, difficulties, and support in the family, we used the social stress and support interview.[15 16] This inquires from the mother abou current stresses and supports in several domains– work, finances, housing, social life with relative and friend, marriage, and children. The degree stress and support in each of these areas is rated on four point scale and total psychosocial stress an supports scores are computed.

INFORMATION FROM THE PAEDIATRICIAN

Paediatricians were asked to note the referring co plaint, their diagnosis, and comments about an associated psychological problems to the presenta tions. The reasons for consultation were classifie according to the International Classification

Health Problems in Primary Care,[17] which generally correspond with ICD 9 scores.[13]

In the analysis of data, tests of statistical significance were used as appropriate (χ^2 with Yates's correction in fourfold tables and non-parametric tests for continuous variables).

Results

The main characteristics of the sample entering the study have been reported elsewhere.[18] A total of 137 parents of children aged 7 to 12 years were approached for this study and 128 (93%) agreed to take part and completed questionnaires. Subsequently two parents declined to be interviewed and 126 interviews were therefore completed. Paediatricians completed questionnaires for 127 subjects and teacher questionnaires were obtained for 100 subjects.

Of the 128 children entering the study there were 66 boys and 62 girls and the mean age for the group was 9·1 years. Most children (103, 80%) were taken to the hospital by their mothers. Most (120, 93%) had been referred by their general practitioners and few had been seen by a medical specialist previously (19, 15%). The main paediatric diagnoses, accounting for half the presentations were: migraine, asthma, abdominal pains, urinary tract infections, growth failure, bedwetting, blackouts, headaches, and soiling. The rest of the children were given one of another 34 diagnoses. Most complaints were long standing and episodic. Mental health problems (that is, behaviour, disorder, anxiety, nigthmares) were the main referring reason in four (3%).

PSYCHIATRIC DISORDER ASSESSED FROM PARENTAL INTERVIEWS

From the information obtained in the psychiatric interview with the parent, psychiatric disorders were judged to be present in 36 (28%) of the children. Disturbed children did not differ from the rest in age: mean (SD) ages were 8·7 (1·6) and 9·3 (1·5) respectively, or in sex distribution.

By far the most common psychiatric diagnosis (present in two thirds of disturbed children) was emotional disorder. Other diagnoses included conduct disorder (5/36, 14%), mixed conduct/emotional disorder (six, 17%) and hyperkinetic syndrome (three, 8%). In two thirds of disturbed children the problems were reported both at home and in school, but in one third they were confined to the home. Most problems were long standing: in over 90% they had lasted more than a year and in two thirds more than three years. The disturbance was judged to be particularly severe (symptoms rated as severe and highly handicapping or multiple) in one fifth of

Table 1 *Psychiatric symptoms in disturbed children: emotional symptoms and relationship problems*

	Symptoms (1, mild or 2, pronounced)	*Disturbed (n=36)*	*Non-disturbed (n=90)*	*Statistics*
No (%) with emotional symptoms:				
Worrying	1	9 (25)	33 (36)	χ^2=12·8 2df p<0·01
	2	14 (38)	10 (11)	
Fears	1	8 (22)	24 (26)	χ^2=15·7 2df p<0·001
	2	22 (61)	23 (25)	
Irritability	1	19 (52)	69 (76)	χ^2=21·6 2df p<0·001
	2	15 (41)	6 (6)	
Chronic unhappiness:				
Episodic		5 (13)	13 (14)	χ^2=11·5 2df p<0·01
Persistent		7 (19)	2 (2)	
No (%) with problems in relationships:				
With mother	1	10 (27)	17 (18)	χ^2=33·1 2df p<0·001
	2	15 (41)	4 (4)	
With siblings	1	10 (27)	19 (21)	χ^2=35·2 2df p<0·001
	2	14 (38)	2 (2)	
With peers	1	10 (27)	11 (12)	χ^2=35·4 2df p<0·0001
	2	13 (36)	2 (2)	
With teachers	1	5 (13)	6 (6)	χ^2=15·3 2df p<0·001
	2	5 (13)	—	

Table 2 *Psychiatric symptoms in disturbed children: activity and antisocial behaviour*

	Symptoms (1, mild or 2, pronounced)	*Disturbed (n=36)*	*Non-disturbed (n=90)*	*Statistics*
Activity—No (%) who are:				
Overactive at home	1	4 (11)	13 (14)	$\chi^2=14\cdot5$ 2df
	2	11 (30)	5 (5)	$p<0\cdot001$
Restless at home	1	6 (16)	19 (21)	$\chi^2=39\cdot3$
	2	20 (53)	8 (8)	2df $p<0\cdot00$
Restless elsewhere	1	3 (8)	2 (2)	$\chi^2=12\cdot2$ 2df
	2	5 (13)	1 (1)	$p<0\cdot01$
Antisocial behaviour:				
No (%) who are stealing	1	4 (11)	3 (3)	$\chi^2=16\cdot6$ 2df
	2	5 (13)	—	$p<0\cdot001$

the children with the rest having less severe difficulties. Most parents of disturbed children (two thirds) said that they thought that their children had emotional or behavioural difficulties but 10 (28%) felt they had no problems.

The psychiatric symptoms that differentiated disturbed from non-disturbed children at high significant levels ($p<0\cdot01$) are given in tables 1 and 2. They included fears, worrying and irritability, unhappiness, problems in relationships (table 1), overactivity, restlessness, and stealing (table 2). Additional symptoms present significantly more commonly in the disturbed group ($p<0\cdot05$) were tantrums, loneliness, obsessionality, worrying about health, problems in the relationship with fathers, overactivity outside the home, aggression, destructiveness, and lying. School refusal was exclusively present in a minority of disturbed children (mildly in five (13%) and pronounced in one (2%)).

In line with the findings from the psychiatric interviews, both the parental and school behavioural questionnaire scores discriminated disturbed and non-disturbed children (parental mean (SD) scores of 16·1 (6·7) and 9·5 (4·7) respectively, $p<0\cdot0001$; teacher mean scores of 9·4 (7·5) and 3·8 (3·8), $p<0\cdot001$). High scores indicative of psychological deviance were obtained in 46 (35%) (parental questionnaires) and 23/100 (23%) (teacher questionnaire) of the children.

Although both the psychiatric interview with parents and the parental behavioural questionnaires used parents as informants, they did not necessarily identify the same children as disturbed. Thus the predictive value of the questionnaire (children identified as disturbed who turned out to be disturbed also from the interview assessments) was 59%. Its sensitivity (disturbed children from interviews also identified by the questionnaires) was 70% and it specificity (non-disturbed children from interview identified as disturbed by the questionnaires) 20%

Prior help for psychiatric difficulties was th exception rather than the rule and only four (11% disturbed children had had psychiatric contac However, three (8%) had consulted their gener practitioner for psychological symptoms in the thre months before assessment.

ASSOCIATION BETWEEN PSYCHIATRIC DISORDER AND SOMATIC SYMPTOMS

As most disturbed children (27, 75%) consulted fo physical problems, we were interested in examinin whether certain physical diagnoses were more fre quent in the disturbed group. We also wished t examine from the child health forms whether dis turbed children had experienced more recen somatic complaints and had more recent conta with medical services. Our results of this analys were mostly negative. The only illness feature t discriminate the two groups was level of energy: 6 out of 90 (68%) of non-disturbed children ha normal energy as compared with 15 out of 36 (41% disturbed children. Of those disturbed 13 (36%) ha high and eight (22%) low energy levels, while fo the rest of the children the numbers were 20 (22% and two (2%) respectively with an additional tw children having variable energy levels reporte ($\chi^2=11\cdot4$, 3 df, $p=0\cdot009$). There was also a trend fo more parents of disturbed children to think that th children's somatic symptoms were aggravated by th child being under psychological pressure (for exam ple, schoolwork or exams: four (11%) compare with two (2%)) and by upsets (for example, worr anger or trouble: eight (22%) compared with 1 (12%)).

Table 3 *Psychosocial aspects in children with psychiatric disorder*

	Disturbed (n=36)	*Non-disturbed (n=90)*	*Statistics*
Current family stress:			
Mean (SD) stress scores	6·3 (3·1)	4·3 (3·5)	Mann-Whitney p=0·001
No (%) with financial stress	12 (33)	12 (13)	χ^2=5·4 1df p<0·05
No (%) with marital arguments:			
Minor	14 (43)	46 (55)	χ^2=5·9 2df
Serious	10 (31)	10 (12)	p=0·05
No (%) with stress concerning children:			
High stress about index child	24 (66)	33 (36)	χ^2=8·1 1df p<0·02
Problems controlling children	22 (61)	18 (20)	χ^2=8·2 1df p<0·01
Low support from children (non-index)	7 (21)	4 (5)	χ^2=5·5 1df p<0·02

PSYCHOSOCIAL BACKGROUND IN DISTURBED CHILDREN

Psychosocial disturbance, particularly a disturbed family background, tends to be an associated feature of childhood psychiatric disturbance. We were interested in establishing which specific psychological problems are of relevance for childhood psychiatric disturbances as it presents in the general paediatric setting.

There were no significant socioeconomic differences between disturbed and non-disturbed children, although unemployment of parents was slightly more common in the disturbed group (nine (25%) compared with nine (10%)). But more disturbed children came from broken homes (14 (39%) compared with 15 (17%); χ^2=5·9 1df; p=0·01), their mothers reported current 'nerve' problems more often (10 (27%) compared with six (6%); χ^2=8·5 1df, p<0·01), they had higher scores on the general health questionnaire (mean (SD) 4·3 (5·9) compared with 1·8 (3·9), p=0·01), and fewer mothers were in outside employment (11/32 (34%) compared with 61/84 (72%); χ^2=10 1df, p<0·01).

At the time of assessment mothers in the disturbed group felt under more general psychosocial stress as measured in the social stress and support interview (table 3), and they noted more financial stress and marital difficulties. Out of the four psychosocial areas explored in this interview, however, most stress was scored by the mothers in relation to index children; they reported more problems in controlling the children and they were experiencing less support from them.

Discussion

Using detailed parental interviews to assess child psychiatric disorder, we found this to be present in 28% of children aged 7 to 12 years referred to general paediatric clinics. Previous studies and our own data from parental questionnaire information have reported slightly higher rates (35% on the Rutter parental scale A[9 10]). This, together with the modest predictive value of the questionnaires, indicates a trend for them to overestimate disturbance in paediatric samples.

Our rates of disorder from the parental interviews were appreciably higher than in general population samples of children of comparable age and psychosocial backgrounds. Thus in the Isle of Wight study, Rutter *et al* identified 12% of all children as psychiatrically disturbed.[19] This in itself strongly suggests that disturbed children are more likely to attend paediatric clinics than non-disturbed children. The high rates in our sample are close to those reported for adult patients admitted to medical wards,[8] and they are only marginally higher than for children attending general practice[11]; this indicates that the link between psychiatric disturbance and medical help seeking is of comparable order in primary and secondary medical settings and shows similarities across the age range.

By far the most common psychiatric diagnosis in our sample was emotional disorder, thus confirming a special affinity between emotional—as opposed to antisocial—and physical symptoms.[11] Inquiry about symptoms such as worrying, fears, irritability, and problems in relationships with mothers, siblings, and peers as well as about restlessness and overactivity is likely to be productive in assessing psychiatric disturbance at the paediatric clinic. Some of the other emotional symptoms present in the disturbed group (obsessionality, worrying about health, school refusal) are of interest because they are quite uncommon among disturbed children in other settings such as children attending child psychiatric clinics (data from the study reported in Garralda and Bailey[20]) and may be specifically related to a 'psychosomatic' tendency to overemphasise somatic over psychological symptoms as a response to stress or difficulty. Similarly long psychiatric histories were particularly conspicuous in this sample and this may indicate that the psychiatric disturbance may be an exaggeration of temperamental difficulties or lifelong traits perhaps associated with a 'psychosomatic' tendency in some of these children.

We found few differences between disturbed and non-disturbed children in types of presenting physical complaints, nor were there difficulties in background physical conditions or in recent contacts with medical services. The only difference was in the global and non-specific health measure of energy level: disturbed children were not only more commonly described as 'bounding with energy', but they were also more apathetic or lethargic than the rest. Energy level stands out therefore as a symptom which may alert paediatricians to the presence of childhood psychiatric disorder.

In terms of the psychosocial associations of psychiatric disorder, our sample was comparable with those in general population studies.[21] Broken homes, current maternal mental health problems, and marital difficulties were more common in the disturbed group. This highlights the importance that these issues and their possible influence on the children's psychological and physical health be recognised as part of the global paediatric assessment.

As in our general practice study,[11] we also found an association between disturbance and family stress. Family stress has been previously found to be associated with increased consultations for children's complaints.[22 23] In the present study the general stress felt by mothers in the disturbed group about their children was higher than for other psychosocial areas explored and difficulties in controlling the children were a major concern for them. This is likely to have contributed to the consultations by sensitising parents to the somatic symptoms and by decreasing their confidence in dealing with the problem or in controlling the situation.

There is evidence that high levels of encouragement and empathy by paediatricians lead to both satisfaction and reduction in concern in mothers.[24] Specific empathic and encouraging statements by doctors could also help boost the mother's confidence and would be especially appropriate for parents of disturbed children attending general paediatric clinics.

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References

1 Coleman JV, Patrick DL, Baker SM. The mental health of children in an HMO program. *J Pediatr* 1977;**91**:150–3.

2 Jacobson AM, Goldberg ID, Burns BJ, Hoeper EW, Hankin JR, Hewitt K. Diagnosed mental disorder in children and use of health services in four organized health care settings. *Am J Psychiatry* 1988;**137**:559–65.

3 Starfield B, Gross E, Wood M, *et al.* Psychosocial and psychosomatic diagnoses in primary care of children. *Pediatrics* 1980;**66**:159–67.

4 Goldberg ID, Regier DA, McInerny TK, Pless IB, Roghmann KJ. The role of the pediatrician in the delivery of mental health services to children. *Pediatrics* 1979;**63**:898–909.

5 Goldberg ID, Claus J, Roghmann J, McInerny TK, Burke JD. Mental health problems among children seen in pediatric practice: prevalence and management. *Pediatrics* 1984;**73**:278–93.

6 McClune D, Richardson MM, Powell JA. Psychosocial health issues in paediatric practices: parents, knowledge and concerns. *Pediatrics* 1984;**74**:183–90.

7 Starfield B, Borkowf S. Physician's recognition of complaints made by parents about their children's health. *Pediatrics* 1969;**43**:168–72.

8 Nabarro J. Unrecognised psychiatric illness in medical patients. *Br Med J* 1984;**289**:635–6.

9 Fitzgerald M. Behavioural deviance and maternal depressive symptoms in paediatric outpatients. *Arch Dis Child* 1985;**60**:560–2.

10 Cundall DB. Children and mothers at clinics: who is disturbed? *Arch Dis Child* 1987;**62**:820–4.

11 Garralda ME, Bailey D. Children with psychiatric disorders in primary care. *J Child Psychol Psychiatry* 1986;**27**:611–24.

12 Rutter M, Tizard J, Whitmore K, eds. *Education, health and behaviour.* London: Longman, 1970.

13 World Health Organisation. *International classification of diseases.* 9th revision. Geneva: WHO, 1975.

14 Goldberg D. *Manual of the general health questionnaire.* Windsor: National Foundation of Education Research, 1978.

15 Jenkins R, Mann HA, Belsey E. The background, design and use of a short interview to assess social stress and support in research and clinical settings. *Soc Sci Med* 1981;**15**:195–203.

16 Bailey D, Garralda ME. The use of the social stress and support interview in families with deviant children: methodological issues. *Soc Psychiatry* 1987;**22**:209–15.

17 World Organization of National Colleges, Academies, and Academic Associations of General Practitioners/Family Physicians. *International classification of health problems in primary care (ICHPPC-2 defined.)* 3rd ed. Oxford: Oxford University Press, 1983.

18 Garralda ME, Bailey D. Psychosomatic problems in the general paediatric clinic. *J Psychosom Res* (in press).

19 Rutter M, Cox A, Tupling C, Berger M, Yule W. Attainment and adjustment in two geographical areas. I. The prevalence of psychiatric disorder. *Br J Psychiatry* 1975;**126**:493–509.

[20] Garralda ME, Bailey D. Child and family factors associated with referral to child psychiatrists. *Br J Psychiatry* 1988;**153**:81–9.

[21] Wolkind S, Rutter M. Separation, loss and family relationships. In: Rutter M, Hersov LA, eds. *Child and adolescent psychiatry, modern approaches*. 2nd ed. Oxford: Blackwell Scientific Publications, 1985:.

[22] Mechanic D. The influence of mothers on their children's health attitudes and behaviour. *Pediatrics* 1964;**33**:444–53.

[23] Roghmann KJ, Haggerty RJ. Family stress and the use of health services. *Int J Epidemiol* 1972;**1**:279–86.

[24] Wasserman RC, Barriatua RD, Carter WB, Lippincott BA. Pediatric clinician's support for parents makes a difference: an outcome based analysis of clinician parent interaction. *Pediatrics* 1984;**74**:1047–53.

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The anatomy of discovery

Preventing rhesus babies: the Liverpool research and follow up

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Nowadays it is 'new genetics' that is all the rage, but it is useful to remember that in the 1950s (when David Weatherall was my house physician) that there was also novelty; then, however, it was blood groups and diseases, and it was that research that helped to interest clinicians in genetics, because the disorders studied were common ones.

Aird, Fraser Roberts, and Bentall were among the new names, and there was much discussion about carcinoma of the stomach and blood group A, duodenal ulcer and blood group O, and what it could all mean.

At about this time the Liverpool genetics group came into being in a roundabout way, and here I must digress to explain the background.

The personal story

As a child I was an ardent butterfly collector, but the urge died away during adolescence only to reappear 20 years later when I spent the last year of the war at a naval hospital in Australia. There the sun shone and the butterflies flew and back it all came. Settling in Liverpool in 1946 I found a way of making butterflies mate ('hand-pairing'[1]) and this led to an interest in hybridisation and then to genetics.

Next I had a lucky break, meeting (in answer to an advertisement in an entomological paper) a young ex RAF geneticist working in EB Ford's department in Oxford—PM Sheppard. We decided to collaborate, firstly on the genetics of hybrids and later to study the evolution of mimicry in tropical butterflies. The work prospered by correspondence, but in the 1950s there was a vacancy for a lecturer in genetics at the University of Liverpool. Philip asked me about this, but, knowing a negative baby when I saw one, I was discouraging and said I thought Liverpool might not suit him. Three weeks later I had a telephone call asking for more information and I knew then that the fish was hooked. He came in 1956 and we collaborated for 20 years, I becoming professor of medicine, and he professor of genetics until he died of leukaemia in 1976.

Soon after his arrival in Liverpool an episode that I remember well was when, motoring down to the Norfolk Broads to look at the swallowtails, I asked him how I could apply my small but increasing knowledge of genetics to medicine. 'Blood groups' he said, and blood groups it was.

Early papers

First came work on blood group O and duodenal ulcer; we disturbed the establishment with a paper that showed that the association disappeared when siblings were used as controls,[2] and later we shot down a paper from the United States[3] that claimed a staggering association between duodenal ulcer and R_1R_2 groups and the MN system, and which had been much acclaimed at the International Congress of Human Genetics in Rome. I had a slight feeling that the findings were too good to be true, and when I showed the paper to our chief technician, Mr WTA Donohoe, he smiled and said: 'Sir' (remember, it was 30 years ago) 'this is a transfusion effect' and when we took patients with ulcers who were not admitted to hospital and whose lesions had not bled the association completely disappeared.[4]

The approach to Rh

It was, however, butterfly mimicry that led to an interest in Rh blood groups, because the method of inheritance of Rh and of the mimetic patterns was rather similar. Rh had three loci (on chromosome 1) Dd, Cc, and Ee, with supposed crossing over to account for the rarer genotypes. The butterflies, too, had a series of genes on the same chromosome ('linked genes'), usually inherited as a unit (a supergene), and we found occasional crossing over giving rise to the rarer patterns.[5] Another parallel was in genetic interactions between the Rh and the ABO systems (ABO incompatibility protects) and sex in the butterflies (only the females have the mimetic patterns).

A natural protective mechanism

And so to the protection against Rh immunisation afforded by ABO incompatibility between mother and fetus. Levine had made the observation that among Rh negative mothers who had produced rhesus babies the incidence of ABO incompatible matings (for example, mother O and father A) was lower than expected.[6] Following this, Race and Sanger suggested that ABO incompatibility as between mother and fetus might actually be protective, the naturally occurring anti-A or anti-B eliminating the fetal cells before they had time to stimulate the production of rhesus antibodies.[7] The experimental work of Stern *et al* supported this hypothesis[8]; they injected Rh positive blood into Rh negative male volunteers and noted that much higher titres of anti-D were obtained when compatible blood was injected than when incompatible blood was injected.

It must be remembered that in an ABO incompatible mating the baby may be compatible, for example, a group O woman married to a man who typed as group A can produce an ABO compatible baby as her husband may be heterozygous AO. Nevanlinna and Vainio pursued this line of research by investigating families in which rhesus haemolytic disease had occurred despite ABO incompatibility between mother and father, and they showed that the immunising fetus was indeed always ABO compatible.[9] In addition, they showed by a family study that once immunisation had occurred ABO incompatibility no longer protected.

The Liverpool team decided to repeat this work and, after studying 91 rhesus families, arrived at a similar conclusion—with the additional finding that the ratio of O X A to A X O mating was 16:33, whereas a 1:1 ratio would be expected if no protection existed.[10]

This work was the beginning of Dr Ronald Finn's MD thesis and the team (usually at that time Clarke, Finn, McConnell, and Sheppard, and a little later John Woodrow and Shona Towers) met frequently to discuss results and future plans, and gradually our interest switched to considering the 80% of Rh negative women who were ABO compatible and therefore at risk for Rh problems if they had a Rh positive baby. Could there be a way of mimicking the protection afforded by ABO incompatibility by giving an appropriate rhesus antibody to the mother after delivery? It sounded good, but could the risks of immunisation be assessed more accurately? Providentially, Zipursky *et al* produced the answer.[11] They applied the technique of Kleihauer *et al*, to detect fetal cells in the maternal circulation.[12] It therefore became possible to assess the size and timing of those transplacental haemorrhages that caused immunisation, and this became a most important additional part of Ronald Finn's thesis. The team also made the important observations that a transplacental haemorrhage occurred usually at or near delivery, and that the larger it was the greater the risk of the mother becoming immunised.[13 14]

In 1960, as secretary of the Liverpool Medical Institution, I organised a symposium on the genetic work being carried out in the department of medicine and Ronald Finn was naturally allotted 'Rh'. I summarised what he said and sent it to the *Lancet*, and it was published three weeks after the meeting, on 5 March 1960.[15] The following is an extract:

> 'Using a modification of the Zipursky method for the detection of foetal red cells, Dr. Finn had investigated the blood of 50 mothers within 72 hours of delivery. Foetal cells were found in about a third of the compatible matings, but were never found in incompatible matings. This finding further supported the hypothesis that ABO incompatibility is protective.
>
> Dr. Finn tentatively suggested that it might be possible to destroy any foetal red cells found in the maternal circulation following delivery by means of a suitable antibody. If successful, this would prevent the development of erythroblastosis, so mimicking the natural protection afforded by ABO incompatibility.'

At this time no one worried about publishing abstracts that had not been out to referees—it never crossed my mind—all we wanted was priority over the workers in the United States who were on our tails.[16]

Trials in volunteers

And so to trying out a 'suitable antibody' in real life. But first, was the research ethical?

In the 1960s there were no ethics committees, but we each had a conscience (I have never seen the word in an ethics committee report). We took advice from people like Robert Race and William Maycock. They both thought that our experimental approach, first of all in men, was correct and that we should have little difficulty in finding volunteers because if the prophylaxis was successful the benefits were obviously going to be great. Dermot Lehane, our Irish Roman Catholic Blood Transfusion Director, excelled with the volunteers. Within a short time we had our 40 Rh negative men from the Liverpool Police Force—'Men of Merseyside mothers-to-be' as the *Liverpool Daily Post* called them. Dermot explained to them every possible risk, including leukaemia from the tagged Rh positive red cells, but to a man they replied (and this really dates us), 'Doctor, we trust you'. Compensation was not mentioned, but Dermot knew how to deal with a minor injection reaction and simply asked, 'How long were you off work, and what do we owe you?' The money was released from a top drawer in Dermot's desk—no forms were needed and we had no problems.

Table 1 *Percentage of cells labelled with ^{51}Cr remaining at various times after injection*

Volunteers	*2 Days*	*5 Days*	*14 Days*
Unprotected	106	88	69
	94	85	67
	98	87	77
Anti-D given	48	33	22
	40	30	21
	13	6	4·6

From Finn *et al*[13] reprinted by permission of the publisher.

The anti-D serum that we decided to use was of the complete type and had a titre with the donor's cells in saline of 1/64. The injection was 10 ml given intravenously.[13] It was Robert Race who advised us to give the 19S because it was this type of antibody that was thought to be protective in ABO incompatibility. Table 1 shows the clearance values in the six volunteers and it will be seen that about 60% of the cells were removed. We waited anxiously and then—to our extreme disappointment—immune antibody production was not suppressed, and there was even a hint that it was enhanced, and our detractors said 'We told you so'. But our mourning was short lived, for even while our 1961 paper[13] was still being written, Ruth Sanger came back from the United States with the news that Kurt Stern and Maya Berger had found that, when Rh negative recipients were injected with Rh positive ABO compatible red cells that had previously been coated with 7S antibody, in vitro, they did not become sensitised to Rh even after repeated exposure to the antigen.[8] We immediately tried this out in our volunteers and found that using 35 to 50 ml of plasma containing predominantly incomplete Rh antibody, three out of 21 'treated' men developed immune antibody compared with 11 out of 21 controls (p=0·02).[14]

These and similar experiments suggested that to prevent immunisation it was necessary to clear about 95% of the injected Rh positive cells within 24 hours, and that the best results were obtained with exclusively 7S anti-D antibody, and much more

Table 2 *Combined results from various centres. Immune antibody results six months and later in controls and after injection of anti-D gammaglobulin*

	Control				*Treated*			*Total in study*
	Total	*Immunised*	*Not Immunised*	*% Immunised*	*Total*	*Immunised*	*Not Immunised*	
Liverpool group (first trial)	136	29	107	21	131	1¶	130	267
Germany*	76	3	73	4	76	0	76	152
Columbia, New York†	113	14	99	12	163	0	163	276
Long Beach, California‡	176	21	155	12	176	0	176	352
Cornell, New York§	58	5	53	9	41	0	41	99
Edinburgh‖	40	3	37	8	41	0	41	81
Totals	559	75	524	13	628	1	627	1227

*Schneider and Preisler[20] and Schneider (personal communication).

†‡§‖Robertson (personal communication).

¶Though there is no doubt about the complete failure in this case, it may be instructive. The patient developed a sharp local reaction to the anti-D gammaglobulin, and we then learnt that she had received early in pregnancy a prophylactic dose of antirubella gammaglobulin. Whether this has any bearing on the failure is uncertain.

From Clarke[19] reprinted by permission of the publisher.

conveniently and safely (no risk of jaundice) with 7S anti-D gammaglobulin, given intramuscularly into the deltoid muscle, rather than with plasma.[16]

The next step was to see if similar results were obtained in Rh negative, postmenopausal women, and this was indeed the case. Success was now in the air and trials of the prophylaxis were taken up by other countries with similar results. This was most encouraging and a period of great international cooperation followed.

Clinical trials

In May 1964 we decided to give, within 48 hours of delivery, 5 ml of gammaglobulin containing incomplete anti-D, usually with a titre of between 1/1280 and 1/4096 in albumin with R_2r cells (later[17] 1 ml was shown to be sufficient). This was given intramuscularly to alternate primiparous women in whom the Kleihauer fetal cell score was 5 or more (the 'high risk' trial) and we decided to analyse this trial when about 70 controls and 70 treated women had been followed up for about six months. The results were highly satisfactory. Of the 78 treated, none had definitely produced immune antibody, whereas 19 of the 78 controls had done so.[18,19] Similar trials (not always restricted to primiparous women and to high risk cases—fetal cell score over 5) were carried out in Germany,[20] the United States, and Scotland, and table 2 shows the overall results, which were excellent.[18]

There was always the possibility, however, that the prophylactic anti-D was only masking immunisation, and that in the next pregnancy the treated woman would produce overt antibody and catch up with the controls. The data in table 3 show that this was not the case.[19]

The result of the Liverpool 'low risk' trial[21] makes an interesting comparison with the final outcome of the 'high risk' one. Although the results of treatment in the low risk trial were good, the incidence of immunisation in the second Rh positive pregnancy was slightly (but not significantly) higher than in the high risk trial. The reason for this is probably that 'primed' mothers are more likely to get into the low risk trial because the number of fetal cells may be low, or even zero, because of concealed antibody that eliminates them. This may also be responsible for most of the failures of anti-D prophylaxis (see table 4).

In those centres where antenatal anti-D is given at 28 and 34 weeks as well as postnatally, this trouble has been circumvented and the incidence of maternal immunisation is considerably reduced.[22] This entails obtaining much more gammaglobulin, however, and the injections can be wasted as the fetus is not usually grouped in utero and the dose is sometimes given when the child is Rh negative. A good compromise would be to give antenatal anti-D in first pregnancies because these women are unlikely to have been 'primed' already and it would mean therefore that a Rh negative woman could probably have at least two babies free of the disease.

Between the successful initial clinical trials and the introduction of routine anti-D by the DHSS in 1970 there were a number of general comments and leading articles on the subject. For example, Jewkes *et al* in *The Sources of Invention* give an accurate account of how it all began, but add a most interesting sideline, which is worth repeating[23]:

> 'An unusually interesting case of individual initiative was revealed in 1967 when an American doctor, Eugene Hamilton of St. Louis, stood up at one of

Table 3 *Combined results: subsequent pregnancies (all centres) up to June 1967, Rh positive ABO compatible infants*

	Control				*Treated*		
	Total	*Immunised*	*Not immunised*	*% Immunised*	*Total*	*Immunised*	*Not immunised*
Liverpool group (first trial)	26	4	22	15	21	0	21
Germany*	7	3	4	43	9	0	9
Columbia, New York†	14	4	10	29	21	0	21
Long Beach, California‡	14	3	11	21	13	0	13
Cornell, New York§	1	1	0	100	2	0	2
Edinburgh‖	1	0	1	0	0	0	0
Totals	63	15	48	24	66	0	66

*Schneider and Preisler[20] and Schneider (personal communication).

†‡§‖Robertson (personal communication).

From Clarke,[19] reprinted by permission of the publisher.

> Dr. Gorman's lectures and announced that he had started clinical trials in April 1962. He had read the B.M.J. article of May 1961(13) giving the first results of the Liverpool workers, and had been impressed with the logic of the approach. He carefully prepared his own plasma from Rh negative mothers who were severely sensitised and had delivered stillborn foetuses but had no history of hepatitis or other communicable disease. Unknown to other workers in the field, he had for five years given protection with successful results to three times as many mothers as any other of the clinical trials, thus doubling the world figures of proven effectiveness of the treatment'.

Bruce Chown wrote in a leader in the *Canadian Medical Association Journal* in 1967 'In less than 30 years our understanding of rhesus haemolytic disease of the newborn has run full cycle from hypothesis to prevention'.[24] The Australians were quick to decide that treatment should be carried out nationwide (see two leading articles in the *Medical Journal of Australia* in 1967 and 1969).[25] [26] The Germans, Finns, Swedes, and Icelanders were early in the field, and even behind what then really was an iron curtain, the Hungarians in particular and the Muscovites and Bulgarians embarked on the treatment.

For all this international collaboration and parallel work it was necessary to standardise the anti-D gammaglobulin, as described in the 1967 report of the WHO meeting about prophylaxis in Geneva.[27] There Hughes-Jones made a great contribution by describing a method that gave more accurate information about the efficiency of an anti-D antibody than did the titre.[28] This method determined the concentration in μg/ml and the value of the equilibrium constant of any particular anti-D. The equilibrium constant influences the amount of antibody that is required in the plasma to give a similar concentration of anti-D bound to the red cells. Thus with 1 ml of red cells present in the circulation, 21 times as much of a weakly binding antibody was required compared with the strongly binding antibody. When there are 400 ml of cells in the circula-

Table 4 *Classification of deaths, registered by the OPCS as due to haemolytic disease of the fetus or newborn, 1977–87*

Categories 1–4 (deaths due to anti-D)

1 Mother believed to have been immunised by a pregnancy following which she was not given an injection of anti-Rh Ig—category divided into:
  1a immunising pregnancy occurred before 1970 when anti-Rh Ig not widely available
  1b immunising pregnancy occurred from 1970 onwards

2 Immunised *during* first pregnancy (anti-D detected during, or within 7 days following, first pregnancy

3 Immunised despite having been given anti-Rh Ig after one or more previous pregnancies (that is, failure of prophylaxis)

4 Immunised to D by blood transfusion

Nos in categories 1–4, 1977–1987

Category	*1977*	*1978*	*1979*	*1980*	*1981*	*1982*	*1983*	*1984*	*1985*	*1986*	*1987*
1a	53	40	40	31	14	17	4	1	5	8	1
1b	32	28	24	23	12	16	12	11	11	9	9
2	12	11	10	6	6	5	8	3	9	8	7
3	9	7	12	11	9	6	9	9	8	5	10
4	0	2	1	1	0	0	1	1	0	0	0
Total	106	88	87	72	41	44	34	25	33	30	27
Per 100 000 births	18·4	14·6	13·5	10·9	6·1	7·0	5·4	3·9	5·0	4·5	3·9

Category 5 Haemolytic disease due to an antibody other than anti-D (anti-c, anti-Kell, etc)
Category 6 Death not due to haemolytic disease

Nos in categories 5 and 6

	1977	*1978*	*1979*	*1980*	*1981*	*1982*	*1983*	*1984*	*1985*	*1986*	*1987*
Category 5	4	3	3	4	3	4	4	5	4	4	0
Category 6	45	49	21	27	13	19	17	19	15	18	8
Total deaths registered by the OPCS as due to haemolytic disease	155	140	111	103	57	67	55	49	52	52	35

tion, only three times as much weakly binding antibody is required as of the strongly binding one.

An 11 year follow up of rhesus deaths in England and Wales, 1977–87

In 1977 the Royal College of Physicians set up its Research Unit and I was the first Director. One of the early projects was to 'audit' the state of rhesus immunisation and with Professor PL Mollison's help we looked year by year at the number of deaths from rhesus haemolytic disease of the newborn (table 4) to find out how and when the mother had become immunised.[29] The Registrar General kindly supplied us with the death or stillbirth certificates for all the infants certified as having died from haemolytic disease of the newborn. In each case we then wrote to the obstetrician who had been in clinical charge of the mother to ask for permission to borrow the notes. This was always given, though it meant a few journeys when the notes were not allowed to leave the hospital.

Over the period, registered deaths and stillbirths from haemolytic disease of the newborn fell by more than 70%, but this fall occurred mainly between 1977 and 1983 and there has been little change since then. Table 4 shows that this is mainly because deaths in categories 2 and 3 have remained constant. These categories include all cases in which postnatal administration could have been of no avail, either because the woman formed anti-D during her first pregnancy (category 2) or because she became immunised despite being given anti-Rh IgG postnatally (category 3) and here she was probably 'primed' (see low risk trial, above).

Category 6 shows that over the period the number of deaths falsely registered as being caused by haemolytic disease fell substantially and progressively. This fall was evidently caused by the dialogue with the office of Population Censuses and Surveys (OPCS) stimulated by this survey. The small number of deaths (8) in 1987 falsely registered may be contrasted with the figure of 45 in 1977.

The figure shows some other data graphically. Firstly, whereas between 1977 and 1980 almost twice as many of the infants were born dead as were born alive, from then on the numbers were about equal. This change was undoubtedly partly the result of the great advances in intrauterine treatment of haemolytic disease, so that hydropic fetuses that would previously have died in utero were then kept alive—for example, by direct intravascular exchange transfusion.[30] The difference must also be partly a result of the advances in the management of premature infants so that pregnancies can be terminated earlier with a good chance of having a viable fetus.

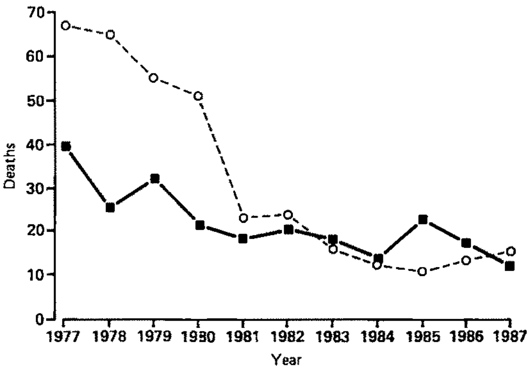

Figure *Number of deaths from rhesus (D) haemolytic disease from 1977–1987. Stillbirths ○——○, children born alive ■——■. (Figure reproduced by courtesy of the editor of the Journal of the Royal College of Physicians of London.[29])*

In this connection it is of interest that the numbers in category 5 changed little in the years 1977 to 1986, whereas one might have expected that there would be an increasing tendency for infants to have been rescued by better obstetric care. The numbers are small, however, but although the figure for 1987 signalled a downward trend, there were three cases of anti-c immunisation in 1988 (unpublished observations). About two thirds of the cases in category 5 were due to anti-c. It has been estimated that about one third of women found to have anti-c during pregnancy have been immunised by an earlier Rh positive (D) transfusion, positive for c, they themselves being C. These cases could be prevented by ensuring that D positive c negative women who had not yet reached the menopause were transfused only with c negative blood. Such blood is readily available from blood transfusion centres.

The fact that stillbirths occurring before the 28th week of pregnancy are not registered means that the OPCS figures and ours underestimate the true mortality for haemolytic disease of the fetus. We previously concluded that our figures might only represent two thirds of the total fetal loss from haemolytic disease. The increasing success of treating severe cases in utero as early as the 20th week, however, implies that the underestimate may be becoming smaller.

It will be interesting to see what the future holds and we plan to continue the survey in Liverpool with the help of Professor POD Pharoah and Dr Ruth Hussey in the department of community health.

Envoi

The Liverpool work is a good example of research starting as 'useless' (our butterflies would certainly not have satisfied 1989 medical audit) but in fact paying off later.[31] It could never have been planned, but we were careful to get expert advice as we went along, and it was encouraging that so many centres in different parts of the world took up the work and kept in close touch so that we knew that our results were repeatable. Nothing in biology is 100% effective, and it is obvious that we have to accept some failures with anti-D, but these are of interest and explicable by the quite unexpected fact that the high risk trial gave better results than the low risk one. Treasure your contradictions!

References

1 Clarke CA. Hand pairing of Papilio machaon in February. *Entomologist's Record* 1952;**64**:98–100.

2 Clarke CA, Edwards JW, Haddock DRW, Howel-Evans AW, McConnell RB, Sheppard PM. ABO blood groups and secretor character in duodenal ulcer: population and sibship studies. *Br Med J* 1956;**ii**:725–31.

3 Buckwalter JA, Tweed GV. The rhesus and MN blood groups and disease. *JAMA* 1962;**179**:479–85.

4 Clarke CA, Donohoe WTA, McConnell RB, Martindale JH, Sheppard PM. Blood groups and disease: previous transfusion as a potential source of error in blood typing. *Br Med J* 1962;**i**:1734–6.

5 Clarke CA, Sheppard PM. Further studies on the genetics of the mimetic butterfly Papilio memnon L. *Philos Trans R Soc Lond (Biol)* 1971;**263**:35–70.

6 Levine P. Serological factors as possible causes in spontaneous abortions. *J Hered* 1943;**34**:71–80.

7 Race RR, Sanger R. *Blood groups in man.* 1st ed. Oxford: Blackwell, 1950:234–6.

8 Stern K, Goodman HS, Berger M. Experimental isoimmunization to hemoantigens in man. *J Immunol* 1961;**87**:189–98.

9 Nevanlinna HR, Vainio T. The influence of mother–child ABO incompatibility on Rh immunisation. *Vox Sang* 1956;**1**:26–34.

10 Clarke CA, Finn R, McConnell RB, Sheppard PM. The protection afforded by ABO incompatibility against erythroblastosis due to rhesus anti-D. *Int Arch Allergy Appl Immunol* 1958;**13**:380.

11 Zipursky A, Hull A, White FD, Israels LG. Foetal erythrocytes in the maternal circulation. *Lancet* 1959;**i**:451–2.

12 Kleihauer E, Braun H, Betke K. Demonstration von fetalem Hämoglobin in den Erythrocyten eines Blutausstrichs. *Klin Wochenschr* 1957;**35**:637–8.

13 Finn R, Clarke CA, Donohoe WTA, *et al.* Experimental studies on the prevention of Rh haemolytic disease. *Br Med J* 1961;**i**:1486–90.

14 Clarke CA, Donohoe WTA, McConnell RB, *et al.* Further experimental studies on the prevention of Rh haemolytic disease. *Br Med J* 1963;**i**:979–84.

15 Finn R. Erythroblastosis. *Lancet* 1960;**i**:526.

16 Freda VJ, Gorman JG, Pollack W. Successful prevention of experimental Rh sensitization in man with an anti-Rh gamma$_2$-globulin antibody preparation. *Transfusion* 1964;**4**:26–32.

17 Report of a Working Party of the Medical Research Council. Controlled trial of various anti-D dosages in suppression of Rh sensitisation following pregnancy. *Br Med J* 1974;**ii**:75–80.

18 A Combined Study from Centres in England and Baltimore. Prevention of Rh haemolytic disease: results of the clinical trial. *Br Med J* 1966;**ii**:907–14.

19 Clarke CA. Prevention of Rh haemolytic disease. *Br Med J* 1967;**iv**:7–12.

20 Schneider VJ, Preisler O. Prevention of Rh sensitization from fetomaternal microtransfusion. *Obstet Gynecol* 1966;**28**:615–21.

21 Woodrow JC, Clarke CA, McConnell RB, Towers SH, Donohoe WTA. Prevention of Rh haemolytic disease: results of the Liverpool 'low risk' clinical trial. *Br Med J* 1971;**ii**:610–2.

22 Tovey LAD, Townley A, Stevenson BJ, Taverner J. The Yorkshire antenatal anti-D immunoglobulin trial in primigravidae. *Lancet* 1983;**ii**:244–9.

23 Jewkes J, Sawers D, Stillerman R. *The sources of invention.* 2nd ed. London: Macmillan, 1969:348–51.

24 Chown B. Prevention of Rh haemolytic disease. *Can Med Assoc J* 1967;**97**:1294–6.

25 Anonymous. Prevention of Rh haemolytic disease. *Med J Aust* 1967;**2**:261–2.

26 Anonymous. Prevention of Rh haemolytic disease. *Med J Aust* 1969;**1**:1035–6.

27 WHO Memorandum. The suppression of Rh immunisation by passively administered human immunoglobulin (IgG) anti-D (anti-Rh°). *Bull WHO* 1967;**36**:467–74.

28 Hughes-Jones NC. The estimation of the concentration and equilibrium constant of anti-D. *Immunology* 1967;**12**:565–71.

29 Clarke CA, Mollison PL. Deaths from Rh haemolytic disease of the fetus and newborn 1977 to 1987. *J R Coll Physicians Lond* 1989;**23**:181–4.

30 Grannum PA, Copel JA, Plaxe SC, Scioscia AL, Hobbins JC. In utero exchange transfusion by direct intravascular injection in severe erythroblastosis fetalis. *N Engl J Med* 1986;**314**:1431–4.

31 Clarke CA, ed. *Rhesus haemolytic disease, selected papers and extracts, with commentaries.* Lancaster: MTP, 1975.

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Current topic

Fever—the fire of life

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The 'fire of life' is the title of Kleiber's book on animal energetics. He wrote of the relation between body size and metabolic rate.[1] The elephant is more economic than the mouse in that it supports its living mass with less energy per unit body weight and thus appears at an advantage. However it is the exposed surface area that determines the environment at which the body works most efficiently, and expressed as energy per unit surface area the mouse has the edge over the elephant. These are mysterious matters of considerable importance to those who produce meat for human consumption. They are of relevance to paediatricians in that newborn infants emerge from one set of circumstances into very different ones, and have to reach a different balance to that of the parents who look after them. If we are to help them when they are sick we have to know of these adjustments, and that was what I proposed to speak of when I chose my title for the Still lecture, 'The fire of life'. But I changed my mind and instead spoke about fever, for it has again become of interest. In this context the 'fire of life' is not the metabolic cost of living but the fever that may cleanse or damage the body. It still allowed me to do what I wish, which is to illustrate an aspect of developmental biology relevant to a current clinical problem.

Is a fever therapeutic or harmful?

This is an old question. The arguments in support are at three levels. Firstly, the high principle; surely such a powerful and consistent reaction, which is seen in poikilothermic as well as homoeothermic animals, must have some survival value or it would not have persisted. Secondly, animal investigations; for example the studies of Kruger and colleagues on the reptile *Dipsosaurus dorsalis* who found that when bacteria were injected the survival was much greater in those which were allowed to select a warmer environment.[2] Thirdly, clinical observations; Rufus of Ephesus (2nd century) observed '... if there were a physician skilled enough to produce a fever, it would be useless to seek any other remedy against disease'; Sydenham (17th century) wrote that 'Fever is a mighty engine which Nature brings into the world to the conquest of her enemies'; Du Bois in his book on fever ended, 'Finally one is faced with the question as to whether or not fever in disease is beneficial. The literature on this subject is extensive and inconclusive' and concluded that 'fever therapy has proved relatively safe and beneficial in many diseases' but that was before the time of antibiotics and steroids.[3]

The alternative arguments run; first on high principle: how can a reaction that places such demands upon the body's reserves enhance its chance of survival? Secondly, animal investigations; it has not been possible to satisfactorily replicate the reptile studies in mammals, and it does not appear to be demonstrable in all lizards either. Clinical observations would suggest that the wide spread use of antipyretics has not been to the subject's disadvantage, but that, in the main, has been after the use of antibiotics.

If high body temperatures assist in the rejection of microscopic invaders then the wide variation in the deep body temperatures found in different mammals and birds takes on a greater interest, does the body temperature determine in part the organisms which colonise or infect us? The table shows typical temperatures.

Clinical issues

(1) Signal of disease

Parents recognise when their child has a fever, they know that to be a sign that their infant is not well, and usually seek medical advice. There are a number of statements in the medical literature from the United States advising parents at what level of temperature they should call their doctor. As part of our aim of increasing parent involvement in the care of their sick child in hospital we have explored the

Table *Average rectal temperature of mammals and birds (for Brody, 1964[4])*

Animal	*Temperature (°C)*
Mammals	
Elephant	36·0
Man	37·0
Rat	37·3
Horse	38·0
Cattle	38·5
Sheep/pig	39·0
Rabbit	39·5
Goat	40·0
Birds	
Turkey, goose, duck, owl, pelican, vulture	40–41
Fowl, pigeon, quail, sparrow, starling	42–43

feasibility of parents recording the temperature; this if nothing else causes one to reconsider just what information can be gained from the temperature, pulse, and respiration charts.

(2) INFECTION IN THE NEWBORN

The newborn infant is subject to infections but rarely reacts with a fever. Theoretically if fever is beneficial then newborn infants like older children might benefit when they have an infection if they were nursed at a slightly higher temperature. It raises the questions as to when and why infants begin to respond to an exogenous pyrogen with a fever.

(3) SUDDEN INFANT DEATH

There is growing evidence that hyperpyrexia might be the final mechanism in at least some infants who die unexpectedly at home.[5] [6] This might be because they are overwrapped and the capacity to lose heat is overpowered. Alternatively it maybe that at the critical age period they develop a response to pyrogen for the first time and generate a fever, which might not be well controlled or may present the infant with a challenge to which they cannot adequately respond because of their thermal environment. The striking statistic of postneonatal infant death in the United Kingdom is that death occurs at a certain age, suggesting a development related factor, and more often in the winter than the summer, which suggests a precipitating infection.

(4) COOL WASHES AND FEBRILE FITS

Why some older infants have a fit when they have a fever is another mystery. The current recommendation to parents of a child who has had a febrile fit is to keep their infant cool with sponging, and to suppress the fever with an antipyretic. Both have been shown to lower the temperature in febrile infants, whether they reduce the incidence of fits is less clear. They are both difficult to administer in time. Cool sponging will increase the infants metabolic rate while antipyretics may reduce it. There ought to be some way of deciding which one is the better approach.

In light of these and other issues now seemed to be an appropriate time to review what is currently known about the development of the response to pyrogen after birth and the development of the thermoregulatory mechanisms, and then to shape our current understanding as a guide to clinical practice and future research.

The response to endotoxin

There are many substances which if injected provoke a fever (exogenous pyrogens[7]). The lipopolysaccharides wrapping bacteria are among the more powerful and are widely used in animal studies. The injection of these endotoxins activates the ubiquitous travelling monocytes or like acting cells which respond by producing interleukin 1. This chemical (or family of similar chemicals) acts at a number of sites.[8] [9] Its action on the brain is to increase the body temperature, encourage sleep, suppress the appetite, and release neuropeptides. Its action on the liver results in the production of the acute phase proteins, the suppression of albumin production, and the inhibition of lipoprotein lipase activity and thus fat utilisation. It enhances the local inflammatory response, and encourages the healing processes by increasing capillary leak, releasing vasodilators, while inducing endothelial cell proliferation and initiating clot formation. But its major effect is perhaps on the haematopoietic and lymphoid cells. It stimulates new cell formation and acts on T cells and provokes the release of interleukin 2, a lymphokine, which activates T and B cell formation. All in all it is a very busy protein.

The weight of its various effects in any one situation may well vary, however taken at its simplest it would seem that the pyrogen effect is linked to all these other activities and a fever properly recorded must be a measure of some of them. The endogenous pyrogen of the older literature is thought to be interleukin 1b.

The production of a fever

Three steps are involved in the development of a fever; first, the invading organism reacts with the phagocytosing monocyte, which in turn releases endogenous pyrogen; second, the endogenous

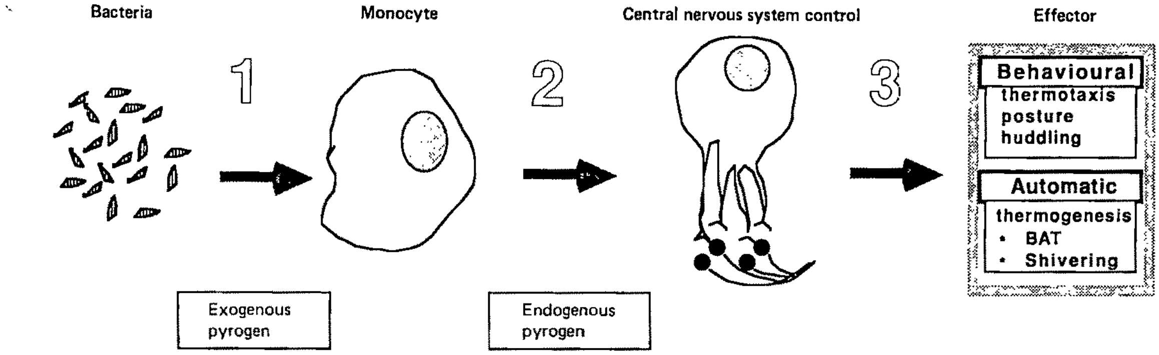

Fig 1 *Three steps in the induction of a fever. BAT=brown adipose tissue.*

pyrogen (interleukin 1b) acts on the central nervous control systems of thermoregulation in the hypothalamus; and third, the thermoregulatory system responds by raising the body temperature, this it may do by a behavioural response—for example, moving to a warmer place, or by an increase in thermoregulatory heat production (fig 1). There is some evidence to suggest that the bacterial material (exogenous pyrogen) might itself act on the central mechanisms and cause a fever, and if the illness causes hypermetabolism then that itself could result in a fever. In most circumstances it seems likely that the three steps are required. Thus if no fever occurs, as may be the case in the newborn, then it might be due to differences in responsiveness at any of the stages in the sequence.

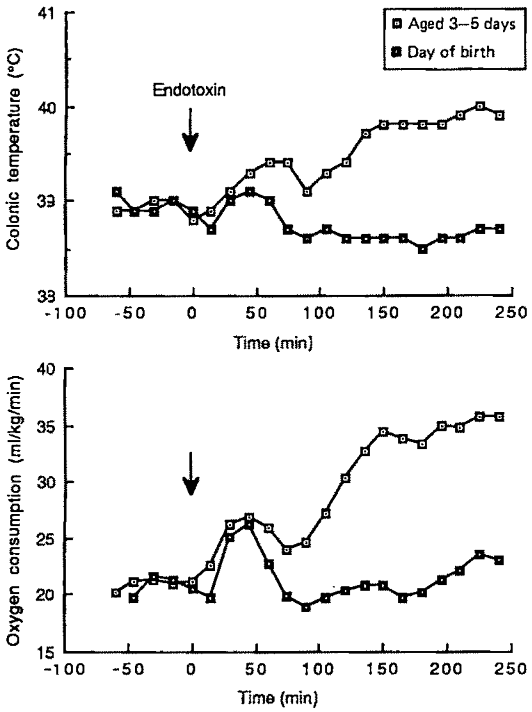

Fig 2 *The mean rates of oxygen consumption and colonic temperatures in rabbits on the day of birth and aged 3–5 days, n=10 (from J Vinter and D Hull, unpublished).*

The development of a febrile response can be demonstrated by injecting endotoxin. Fig 2 shows the mean rise in body temperature and rate of oxygen consumption of rabbits aged 3 to 5 days after intraperitoneal injection of lipopolysaccharide from the shell of *Escherichia coli* bacteria. The rise was followed by a rise in colonic temperature. The rabbits were initially placed in an ambient temperature within their thermoneutral range and this temperature was not changed for the duration of the study. This response is similar to that found in adult rabbits and has two phases, a rise after a latent period of 15 to 20 minutes reaching a peak in 40 to 50 minutes followed by a fall only to begin to rise again in the 60 to 70 minute period. However, similar rabbits on the day of birth showed no such response. These results are similar to those reported earlier by Székely and Szélenyi.[10] Clearly something is developing rapidly after birth, presumably induced by the birth process or the new environment.

Similar findings have been observed in other species—for example, guinea pigs[10 11] and lambs.[12] Thus it would seem that there is some hesitancy after birth in the response to pyrogen but in the guinea pig and lamb as well as rabbits an unequivocal response can be demonstrated on the second or third day of life. It does seem on the evidence that is available that in general the presence or not of the response varies with the dose and that young animals need a relatively larger dose than older animals, but the magnitude of the response, once elicited, does not vary with age and increasing the dose does not increase the magnitude of response.

Behavioural thermoregulatory responses to pyrogen

Endogenous pyrogen acts by adjusting the thermoregulatory response such that a higher body temperature is defended. This may be either by behavioural or automatic responses. The former are preferred because they put less demand on energy reserves. Newborn rabbits show behavioural thermoregulatory responses from the moment of birth, ambient temperature appears to be the most powerful stimulus to their activity, it draws them towards their mother and it draws them towards each other in a cool environment. In a warm environment they separate.[13] Fig 3 shows the behavioural responses of 3 to 5 day old rabbits injected with endotoxin. The rabbits given endotoxin selected a higher environmental temperature.

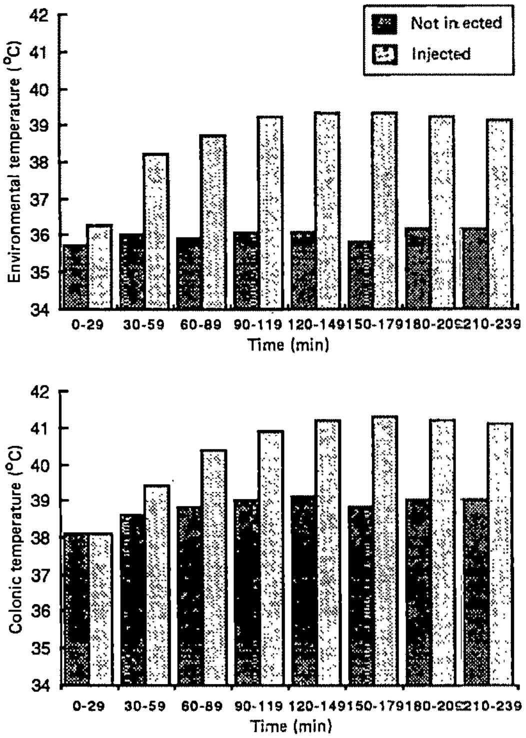

Fig 3 *Selected environmental temperatures and colonic temperatures in rabbits aged 3–5 days in littermates not injected and injected with endotoxin, n=10 (From J Vinter and D Hull, unpublished).*

It is reasonable to ask that if newborn rabbits ar stimulated by pyrogen what behavioural respons might they make? It is interesting that the youn move themselves around within a litter so as t maintain body temperatures, presumably the febril one might act to stay in the midst of the grou longer.

The rabbits injected with endotoxin chose a environment that gave them a higher deep bod temperature than that achieved by the rabbits kep within their original thermoneutral range. Th reasons became clear after more detailed study o the automatic or physiological thermoregulator response.

Automatic or physiological thermoregulatory response to pyrogen

The physiological thermoregulatory adjustments t different environmental temperatures are shown i the format used by Mount (fig 4).[14] The values ar for a term infant, nursed naked, and assumes tha the responses, sweating, vasomotor and postura changes, and thermogenesis proceed in sequenc and do not overlap, and that the deep bod temperature is held at 37° (which in the event it doe not), and that the infants physical characteristic relevant to heat exchanges stay constant.

It is generally suggested that pyrogen resets th thermoregulatory set point upwards. Thus it migh be anticipated that if the individual is exposed t pyrogen, but is held in the same environment, the thermogenesis would increase and that increas would be formed of two components (fig 5). Th first would be thermoregulatory thermogenesis an the second an increase in the minimal rate due to th so called Q_{10} effect. The van't Hoff/Arrheniu relationship states that the rate of a chemic reaction is proportional to the temperature, biochemical reactions have a Q_{10} between 2 and 3, thus fo every degree rise the metabolic rate might b expected to increase 10%. Studies on man and adul animals have shown a rise in metabolic rate with fever. It is not always clear whether thermoregulatory thermogenesis has been excluded and it appear to be a very variable phenomenon. Thus while th metobolic rate of poikilothermic animals may increase with surrounding and body temperatures th evidence that homoeothermic mammals 'obey' th Q_{10} effect remains uncertain.

Making the necessary investigations to establis whether the situation illustrated in fig 5 is the tru one is difficult because of the many interferin variables, activity, feeding, restraint, sleep, etc. I this respect the newborn rabbit, which feeds once day and does little else, is particularly suitable. Fig

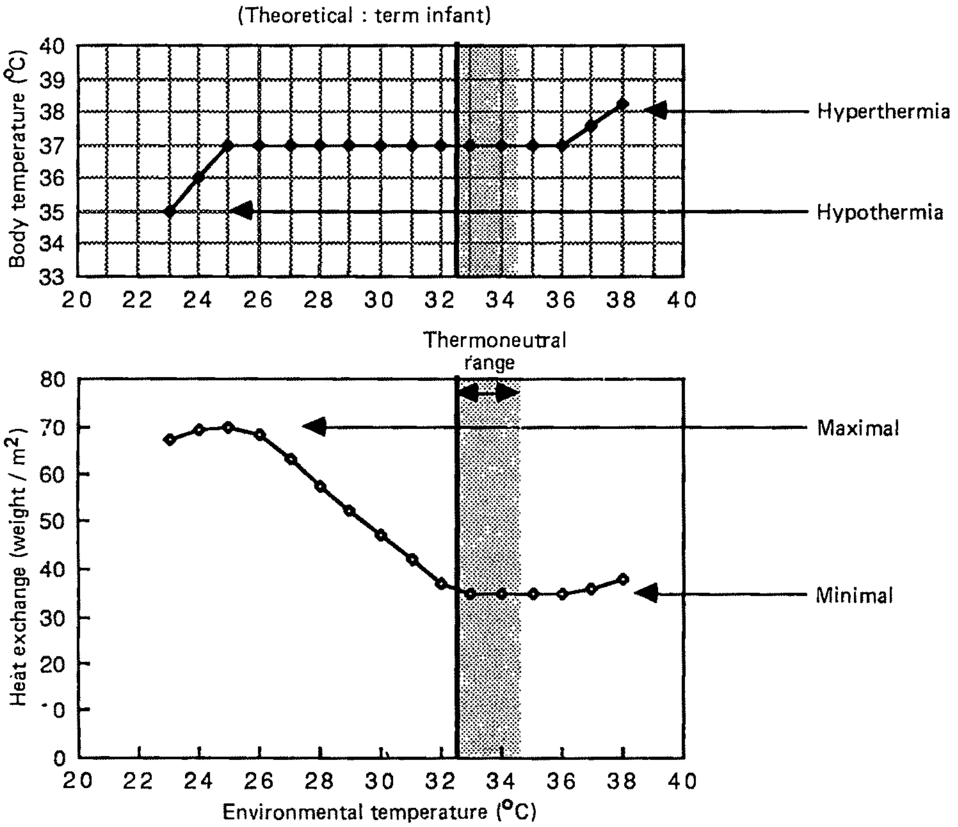

Fig 4 *Theoretical average values of heat exchange and deep body temperature of naked term infants based on best available information and making certain assumptions (see text and Wheldon 1982[15]).*

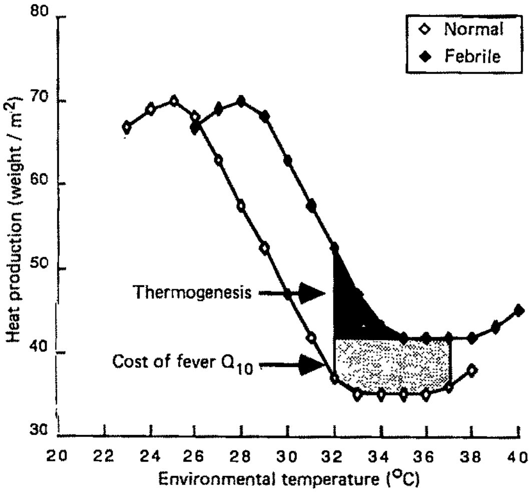

Fig 5 *The theoretical effect of changing the thermoregulatory 'set point' in the normal and febrile infant.*

illustrates the findings on two groups of rabbits aged 3 to 5 days, one of which was given pyrogen before the observations.

The results are of interest for three reasons. Firstly, there was no Q_{10} effect. In other words when the rabbits were in a thermoneutral environment for their new set temperature they did not have an increase in metabolic rate. There is no inevitable cost for a fever. The reason why the rabbits given pyrogen and kept in their original thermoneutral range (fig 1) had an increased metabolic rate is because they then perceived that environmental temperature as cool.

Secondly, the association between metabolic rate with thermal environment did not alter: although the set point was adjusted upwards as shown in fig 5, the gradient changed. The gradient is a measure of thermal insulation which is most easily calculated if the colonic temperature remains constant. But in rabbits, as in newborn infants, the colonic temperature does not stay constant but slides down with the ambient temperature. The reason for this is not clear, but its effect is to allow some thermal control over a wider range of thermal conditions. After administration of endotoxin the gradient in the fall of rectal temperature was greater. The effect of this was that at thermoneutral temperatures the endotoxin resulted in much higher colonic temperatures but the difference with those not given endotoxin decreased as the ambient temperature fell so that endotoxin made no difference to body temperatures at ambient temperatures at the lower end of the range of thermal control.

Thirdly, the change in gradient indicates that the pyrogen acts to influence the gain rather than shift the set point. Two models have been proposed for the central control of temperature and to explain the effects of pyrogen.[16] The first assumes a central mechanism that has some inherent prescribed set temperature that the system defends. The second proposes that pyrogens act by influencing the responsiveness to feed back information and thus affect the body temperatures. If pyrogen acted to change the set point it would produce the theoretical response illustrated in fig 5, if it altered the gain it would in effect produce the response seen in 3 to 5 day old rabbits illustrated in fig 6. Thus if the fever has any benficial effect then it will be most effective in a warm environment when it will make least demand in the new thermoneutral environment.

The practical implication of this is that if the aim of management is to remove the burden of the metabolic response to fever then the appropriate step would be to keep the infant warm. A problem arises with a fluctuating temperature.

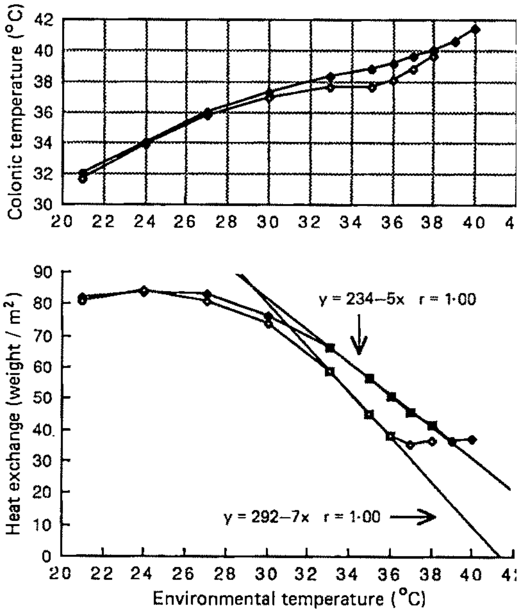

Fig 6 *Mean values of the heat exchange and colonic temperatures of rabbits, 3–5 days old, in control (open symbols) and after injection of endotoxin (closed symbo (J Vinter and D Hull, unpublished).*

Development of thermoregulatory control

The development of fever depends on thermoregulatory control and effective thermoregulatory mechanisms either behavioural or thermoregulatory thermogenesis. In some mammals they are present on the day of birth. But this is not so in all mammals. For example, the hamster on the day of birth is poikilothermic in that it is unable to show any response to changes in environmental temperature and has no control over its own body temperature. The mother controls the temperature of her young by the time she spends in the nest: she is the source of heat as well as insulation. By eight days the young hamster shows thermotaxis, that is it moves to a warm temperature but not in a controlled way, by 12 days it shows effective behavioural thermoregulation, and by 16 days it has developed effective automatic thermoregulatory mechanisms. The development of thermogenesis in the hamster would appear to illustrate the concept popularised by Barcroft and beloved by developmental physiologists, th 'ontogeny follows phylogeny'. What seems clear that the central mechanisms of thermoregulato control develop in advance of the thermogenic effe tor responses. In most newborn mammals th means thermogenesis in brown adipose tissue. some larger mammals and in the guinea pig, rabb and kitten thermoregulatory control (behaviou and automatic) is demonstrable on the first day life. In them the absence of a response to endotox over the first days cannot be explained on the ba that the body could not effect the increase temperature provided the investigation was done an appropriately warm environment. The del would seem to reside in the production of pyrog or its activity centrally.

The human term newborn infant shows therm regulatory thermogenesis on the day of birt though it may increase in magnitude over the fi hours of life. It can also make behavioural respons if allowed. The clinical impression that newbo infants do not react with a fever requires examination when due consideration is given to t environmental temperature. Precise observatio would suggest that a febrile response does devel soon after birth. Observations on newborn lam suggest that previous exposure to endotoxin may accelerate the development of the response.[12]

Immature infants are a different matter, or are they? Certainly they do not develop pyrexias, but is that because they only have a limited thermogenic capacity (none has been found in infants under 28 weeks' gestation though they have brown adipocytes, but some response is seen in infants 32 weeks and over[17]) or is it because they are nursed in such a way that any fever they develop would immediately be corrected by adjustment in their environment such that they strive to increase their temperature but are unable to succeed. Such activity might well present them with an unnecessary metabolic burden.

The human infant remains dependent on the awareness of his or her parents for behavioural reactions for some months after birth, the infant left alone depends on automatic responses. Those at birth are primarily thermoregulatory thermogenesis in the cold and sweating when it is too warm. Although all the sweat glands are present at birth, and sweating on the forehead appears profuse, the infant's capacity to loose extra heat by this mechanism is comparitively limited. Thus as the infant grows at least two changes are occurring: the sweat glands may be becoming more efficient and the infant may be shifting from non-shivering to shivering thermogenesis. The timing of these events is not known.

Conclusions

In answer to the clinical questions poised it would seem that measuring an infant's body temperatures and interpreting them in the light of the environmental conditions may be even more instructive than we thought, we need to discover to what extent it reflects the other activities of interleukin.

In the newborn we might be able to do much more to meet the sick infant's needs by adjusting some of our responses. For example, it would be interesting to know if immature infants with an infection have relatively high metabolic rates and would benefit by being nursed in a warmer environment, and we need to explore the quality of the other aspects of the bodies response to interleukin.

As for hyperthermia in older infants, there may be a time when the thermoregulatory system undergoes critical stages in development and the generation of interleukin may also be a variable and measurable response which might be inducible and possibly open to beneficial influence. Infants are particularly vulnerable to overheating if they experience fluctuating exposure to pyrogens while being nursed in the same thermal conditions.

There can be no doubt that cool washes if they lower the body temperatures will in so doing increase the metabolic rate. Thus if a fever is hazardous and needs to be corrected cool washes, while of value in an emergency, are a poor alternative to an antipyretic.

What Du Bois said forty years ago is still true, *'There is still much to be learned about fever'*.[3]

This is the text of the Still Lecture, which was presented at the British Paediatric Association Annual Meeting in York, April 1989.

References

[1] Kleiber M. *The fire of life and introduction to animal energetics.* New York: John Wiley, 1961.

[2] Kruger MJ. Is fever beneficial? *Yale J Biol Med* 1986;**59**:89–95.

[3] Du Bois EF. *Fever and the regulation of body temperature.* Springfield: CC Thomas, 1948.

[4] Brody S. *Bioenergetics and growth.* New York: Hafner Publishing Company, 1964.

[5] Bacon CJ. Overheating in infancy. *Arch Dis Child* 1983;**58**: 673–4.

[6] Stanton AN. Overheating and cot death. *Lancet* 1984;**ii**: 1199–201.

[7] Dinarello CA, Wolff SM. Exogenous pyrogens. In: *Pyretics and antipyretics.* Milton AS, ed. Berlin: Springer-Verlag, 1982:——.

[8] Dinarello CA. Interleukin 1. *Rev Infect Dis* 1984;**6**:51–95.

[9] Strober W, James SP. The interleukins. *Pediatr Res* 1988;**24**: 549–57.

[10] Székely M, Szélenyi Z. The pathophysiology of fever in the neonate. In: AS Milton, ed. *Pyretics and antipyretics.* Berlin: Springer-Verlag, 1982:——.

[11] Blatteis CM. Postnatal development of pyrogenic sensitivity in guinea pigs. *J Appl Physiol* 1975;**39**:251–7.

[12] Kasting NW, Veale WL, Cooper KE. Development of fever in newborn lambs. *Am J Physiol* 1979;**236**:184–7.

[13] Hull J, Hull D. Behavioural thermoregulation in newborn rabbits. *Journal of Comparative and Physiological Psychology* 1982;**96**:143–7.

[14] Mount LE. *Adaptation to thermal environment.* London: Edward Arnold, 1979.

[15] Wheldon AE. Heat exchange between the newborn baby and the environment. Nottingham: Nottingham University, 1982. (PhD thesis.)

[16] Mitchell D, Snellen JW, Atkins AR. Thermoregulation during fever, change of set point or change of gain. *Pflugers Arch* 1970; **321**:293–302.

[17] Wheldon AE, Hull D. Incubation of very immature infants. *Arch Dis Child* 1983;**58**:504–8.

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Personal practice

Practical management of sickle cell disease

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Sickle cell disease is a common problem in several inner city populations in this country, but the overall incidence is low. It has been estimated that 5000 people in the United Kingdom have the disease, at least 2000 of these living in London alone.[1] Sickle haemoglobin results from a single amino acid substitution in the β globin chain. The disease is inherited and the important sickling syndromes are homozygous sickle cell disease (Hb SS) and the double heterozygotes, haemoglobin SC disease (Hb SC) and sickle β thalassaemia. Hb SC disease and sickle thalassaemia tend to be milder than Hb SS. There is tremendous variability in the severity of sickle cell disease within all these groups. Some patients are plagued by constant hospital admissions with painful crises and subsequent progressive organ damage; other patients are comparatively symptom free and may not be diagnosed until late in life. Persistently high concentrations of fetal haemoglobin are commonly associated with milder disease and the coincident inheritance of α thalassaemia may also mitigate against severe disease. In most patients, however, the factors responsible for severity are not understood, and the disease course may be hard to predict. This has important implications for genetic counselling and community education programmes.

There are no accurate mortality figures for sickle cell disease in this country, but mortality in an American urban population was of the order of 10% in the first decade of life.[2] A Jamaican study showed that the highest mortality was during the first five years of life—10% of cases die in the first year, 5% in the second, and 3% in the third.[3] It is clear that paediatricians carry the burden of responsibility for ensuring survival of patients with sickle cell disease.

Newborn babies with sickle cell disease have normal blood counts because of normal fetal haemoglobin production and sickle cell disease does not usually present clinically until after the first six months of life when fetal haemoglobin has declined towards adult concentrations. The diagnosis can, however, be made at birth if sensitive haemoglobin electrophoresis techniques are used.[4] Because of the high early mortality from potentially preventable or treatable complications it is essential to diagnose patients early. In areas where there is a large population at risk the best method is probably by screening the cord blood of all neonates. In areas with a lower incidence of heterozygote carriers, selective screening of children born to heterozygote mothers may be more appropriate; it is important that the approach is tailored to suit the local population. Once a child is diagnosed as having sickle cell disease, he or she needs a comprehensive programme of care and should be reviewed regularly at a haemoglobinopathy clinic. The diagnosis should be confirmed by repeat testing at three to six months.

Infection

Patients with sickle cell disease are more susceptible to infection than normal subjects, because of hyposplenism, defective opsonisation mechanisms, and other ill defined factors. The organisms to which patients with sickle cell disease are particularly prone include pneumococcus, *Haemophilus influenzae*, and *Salmonella* spp. The risk of infection by pneumococcus is at least 600 times that in the normal population, and the period of greatest risk is within the first three years of life when it is the main cause of death. Continuous prophylactic penicillin in infants aged from 3–36 months results in an 80% reduction in the infection rate, and needs to be started by the age of 4 months and continued at least until adult life.[5] The role of antipneumococcal vaccine in preventing infection is less clear. There is evidence of both a serological response and efficacy in children with sickle cell disease over the age of 2 years, and giving the vaccine at this age may well provide additional protection.[6] The role of the vaccine in children with sickle cell disease less than 2 years of age remains to be defined. Parents should be educated about the risk of infection, and urged to bring children with sickle cell disease who have a fever or who are unwell to hospital for urgent assessment. These young children should be given broad spectrum antibiotics intravenously (including penicillin) without waiting for results of culture. It is always important to look for the source (blood, lungs, bones, meninges, urinary tract) and to treat the infection appropriately. Routine childhood immunisations should be given as usual.

Splenic sequestration

This is the other major cause of mortality in the early years, and may be precipitated by infection. It is characterised by the sudden onset of pallor, breathlessness, abdominal pain, and splenic enlargement. Rapid sequestration of red cells in the spleen leads to sudden anaemia and death. Blood needs to be given without delay and occasionally this may have to be uncrossmatched O negative red cells. It is important to give broad spectrum antibiotics intravenously to cover the pneumococcus and *H influenzae*. Splenic sequestration crises often recur and it is usual to carry out elective splenectomy after the child has recovered from an episode. In children under the age of 2, however, prophylactic red cell transfusion to maintain the level of haemoglobin S below about 30% prevents recurrence and allows a delay in splenectomy until the age of 2 when the risk of postsplenectomy septicaemia becomes less. Parents of young children with sickle cell disease should be warned of the risk of splenic sequestration and taught to look for splenomegaly should the infants become unwell.

Acute painful crisis

This results from vaso-occlusive episodes and may be provoked by infection or dehydration. Pain occurs in any part of the body but is most frequently seen in the bones, muscles, and abdomen. Young children suffer the 'hand-foot' syndrome characterised by infarcts of the metatarsals and metacarpals and painful swelling of the hands and feet. Crises are often painful and debilitating. There is no specific curative treatment but the child should be given fluid replacement, pain relief, and antibiotics.

Many children with sickle cell disease have reduced tubular concentrating ability, and the continuing fluid loss without adequate replacement causes a reduction in plasma volume with increased plasma viscosity and aggravation of sickling. They require roughly 80 ml/kg of fluid in 24 hours and, although an adequate oral intake can sometimes be achieved, it is usually necessary to give intravenous fluids to those with severe pain or abdominal symptoms. Intravenous fluids should be continued until the child is free of pain and taking adequate oral fluids and it is important that the oral intake is maintained even when the pain has gone.

Pain caused by vaso-occlusion in sickle cell disease is frequently severe and often underestimated. The aim should be to reduce the pain fully as soon as the child has been assessed. A dose of an intramuscular opiate provides rapid relief and may be followed by an oral analgesic or an opiate infusion. The risks of opiate addiction seem to be small.

It is always important to look for a focus of infection and treat it appropriately. Any child with a high fever or who appears clinically ill should, however, be treated immediately with intravenous antibiotics (including penicillin). The haemoglobin concentration usually remains steady in painful crises and a falling haemoglobin concentration suggests sequestration in lung, spleen, or liver.

Joint effusions and swelling of tissues over infarcted bone often occurs. In children with sickle cell disease there is also an increased incidence of osteomyelitis, often caused by salmonella, which may be multifocal and difficult to distinguish from infarction. If the child is febrile and there is pronounced inflammation, or if fever and swelling fail to settle, blood cultures should be repeated, a radiograph taken of the affected area, and an orthopaedic opinion sought urgently. Acute surgical problems may mimic abdominal crises, and it is always important to consider a wide differential diagnosis.

Chest syndrome

This is more common in older children and adults, and is characterised by lung consolidation on the chest radiograph, which may be bilateral. Chest pain and breathlessness may occur, but physical signs may be scanty. Children with this syndrome may deteriorate rapidly. Antibiotics should be given intravenously as soon as blood and sputum have been obtained for culture. Transfusion should be given if the haemoglobin concentration is falling, and exchange transfusion may be necessary in severe cases.

Stroke

This is one of the most devastating complications of vaso-occlusion and is an indication for acute exchange transfusion. The risk of recurrence is high and a programme of regular transfusions should be embarked upon to prevent this.[7]

Anaemia

The haemoglobin concentration is reduced in patients with sickle cell disease as a consequence of chronic haemolysis, but remains stable in any individual case. The haemoglobin concentration may be near normal in the SC disease, and children may be misdiagnosed as having sickle trait because of a positive sickling test. It is vital to perform full electrophoresis in all cases. Children do not have symptoms of anaemia, because the shift in the oxygen dissociation curve allows the haemoglobin to give up more oxygen to the tissues. Clinical anaemia may result from various causes including sequestration or reduced marrow activity. All children with sickle cell disease have an increased need of folic acid, and if folate intake is inadequate they may have a megaloblastic crisis as a result of acute folate deficiency. Anaemia may be caused by acute red cell hypoplasia that results from viral infections, particularly by parvovirus. During the period of red cell hypoplasia haemolysis continues unabated, reticulocytes are almost completely absent, and patients may become severely anaemic and require transfusion. Recovery is spontaneous and immunity to the parvovirus lifelong.

Blood transfusion

There is little evidence that blood transfusion during a simple painful crisis either reduces its severity or curtails its length. Repeated transfusions to suppress erythropoiesis and keep the haemoglobin S concentration less than 30% may be used to prevent complications such as splenic sequestration and stroke, and sometimes in preparation for elective operations. In children receiving regular transfusions, iron chelation treatment will become necessary as iron overload begins to develop. Exchange transfusion may be indicated to prevent sickling in acute conditions such as chest syndrome, priapism, or stroke, and occasionally it is necessary in the preparation of a patient for an elective operation. The risks of blood transfusion including reactions, sensitisation, and infection need to be balanced against the potential benefits.

Development

Children with sickle cell disease are usually thin, and their weight tends to be lower at all ages. Puberty is often delayed and the growth spurt is accordingly delayed, but the final height is usually near normal. Enuresis is a common problem, probably as a result of high fluid intakes and urinary volumes, but it eventually resolves.

It is important to manage all these problems with reassurance rather than inappropriate investigation or intervention.

Comprehensive care

One of the most important developments in the management of sickle cell disease has been the recognition of the importance of a comprehensive programme to cover all aspects of health and development, and the development of specialist haemoglobinopathy clinics. In areas with a high incidence of the disease it is now common to employ sickle counsellors to liaise among the medical, social, and educational services. They play a vital part in the health education of affected communities and of the children and families. Some children miss a lot of schooling and it may be necessary to arrange for tuition at home during periods when complications are preventing attendance at school. An adequate standard of housing is probably of great importance in maintaining the health of children with sickle cell disease, cold and damp are likely provokers of crises and stress may also contribute to a poor clinical course. The employment achievements of people with sickle cell disease have been poor[8]; this is at least in part related to poor educational achievement as a result of chronic illness.

Regular clinical review forms an important part of the management of sickle cell disease. Chronic organ damage may become a problem in teenage children and adults; liver, lungs, heart, and kidneys may all be affected. Regular attendance at clinics also provides the opportunity to ensure compliance with penicillin prophylaxis and folic acid treatment.

Antenatal diagnosis

Sickle cell counsellors, supported by the haemoglobinopathy clinic and the laboratory, are beginning to assume a vital role in antenatal diagnosis and counselling. Antenatal diagnosis for sickle cell disease by chorionic villous biopsy is now possible in the first trimester of pregnancy, and it is important that good non-directive counselling that takes account of the variable clinical course is available for affected couples. The recent introduction of haemoglobinopathy cards by the Department of Health reflects increased awareness of the need for community health education. Failure to provide adequate backup by qualified counsellors, however, is likely to prevent success.

Future directions

Despite a clear understanding of the molecular basis of sickle cell disease, the development of specific treatment has been disappointing. Numerous antisickling agents have been tried over the years and have been found to be either ineffective or toxic. Vasoactive drugs and attempts to promote persistence of fetal haemoglobin have been equally unsuccessful.

Bone marrow transplantation has been successfully carried out, but the risks of graft versus host disease and of the marrow ablative treatment required for this approach are too high to allow it to become generally applicable. Gene treatment is viewed with great interest and optimism but it is still far from having a clinical application.

For the time being, management hinges around supporting the child and family and preventing and treating complications.

References

[1] Brozovic M, Anionwu E. Sickle cell disease in Britain. *J Clin Pathol* 1984;**37**:1321–6.

[2] Powars DR. Natural history of sickle cell disease—the first 10 years. *Semin Hematol* 1975;**12**:107–26.

[3] Sergeant CR. Observations on the epidemiology of sickle cell disease. *Trans R Soc Trop Med Hyg* 1981;**75**:228–33.

[4] The British Society for Haematology. Guidelines for haemoglobinopathy screening. *Clin Lab Haematol* 1988;**10**:87–94.

[5] Gaston H, Verter J, Woods G, *et al.* Prophylaxis with oral penicillin in children with sickle cell anaemia: a randomised trial. *N Engl J Med* 1986;**314**:1593–9.

[6] Ammann AJ. Current status of pneumococcal polysaccharide immunisation in patients with sickle cell disease or impaired splenic function. *Am J Pediatr Hematol Oncol* 1982;**4**:301–6.

[7] Powars D, Wilson B, Imbus C, *et al.* The natural history of stroke in sickle cell disease. *Am J Med* 1978;**65**:461–71.

[8] Franklin IM, Atkin K. Employment of persons with sickle cell disease and sickle cell trait. *J Soc Occup Med* 1986;**36**:76–9.

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Personal paper

Geriatric paediatrics

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Interest in the medicine of childhood is shifting from concern with mortality and short term morbidity to the long term effects of childhood ill health on the well being of adults. There is a clear need for very long term studies of the outcome in children exposed to deprivation and illness in early life.

It has always been known that the infant mortality rate is a good index of the well being of a population, as indeed straightforward biological considerations would imply; but only recently have hard data been available linking such rates to the health into old age of the survivors in a definable cohort We now have the work of Barker and Osmond to remind us that, though it may be the fittest who survive disease in childhood, a proportion of the survivors are to some extent rendered unfit for the rest of their lives by having to surmount too testing a challenge at a vulnerable time in their development.[1] In some cases it can even be shown that this relationship applies to particular organ systems—for example, recurrent bronchitis in childhood predisposes to chronic bronchitis in middle age [2]

We do now of course have the lowest mortality rates in childhood that have probably ever been attained in this country as a whole; but nevertheless major class differences persist within our population and our rates are not as low as those of better off or better organised 'neighbours'.[3] To take some obvious examples: nephritis and pyelonephritis acquired in childhood probably account for something like a half of all cases of renal failure requiring dialysis or transplants in adult life.[4–7] The same can be said for chronic valvular heart disease in adults, which used to be the long term consequence of childhood rheumatic fever,[8] while there is a probably justified suspicion that atherosclerosis and hypertension may be the late effects of processes beginning in early life.[9] [10] Yet what we know to be demonstrably so in some instances is more than matched by what we do not know but may suspect—such as Dr Murray's suggestion (coming from no less an authority than the Dean of the Institute of Psychiatry) that there may be a relationship between perinatal insult and schizophrenia.[11] Nor should we forget that our success in prolonging the lives of children suffering from what used to be fatal conditions like diabetes, leukaemia, and cystic fibrosis now presents physicians for adults with quite large numbers of patients whose adult existence will remain medically precarious until better ways are found of treating them. Thus we have the paradox that if we wish to minimise ill health in our increasing population of pensioners, we need to start at their conception, not catch up with their problems in middle age. Moreover, it needs to be pointed out that their pensions will be paid for by the work of the generation treading on their heels, and will depend therefore on *its* physical and psychological well being.

This, of course, is not necessarily an argument for increasing medical and nursing provision for our child population: economic and cultural factors are probably more important in determining their well being.[11] But nevertheless, without study of what it is that relates poverty and ignorance to physical and emotional illness, we shall not succeed in applying resources in a cost effective way, and only *medical* research will enable these relationships to be worked out in sufficient detail for preventive measures to be properly focused. Attributing ill health to inferior genes will only lead us into the morally suspect field of eugenics. Success is more likely to come from the discovery of what is the best environment for a particular genetic constitution—for example, a phenylalanine deficient diet for those with phenylketonuria. It is clear that there is great scope for a widening of the concept of preventative medicine in line with the recognition that the future of medical child care lies in the appointment of more, and better educated and organised, community paediatricians, using hospitals as community resources and concerned with the provision of the best possible environment for successful child rearing by the removal of what gets in its way,[8] and by the identification of special needs.

The purpose of this paper is to put forward the arguments for establishing a Medical Research Council (MRC) unit concerned with very long term studies of the effects of genetic factors,[12] of childhood illness, and of deprivation on the well being of adults and their proper functioning—well defined by Freud as the ability to love and to work: that is, to hold down a job and a marriage. These are the essentials for an enjoyable and creative life, for successful reproduction, and for the maintenance of the nation as a territorial and cultural entity with its particular way of contributing to the well being of the human race as a whole.

If we admit to the need for such long term studies, including those involving planned intervention, it must be clear that they will never be seen through on the basis of short term grants and the relatively short term interests of particular workers or departments, but will need appropriate guaranteed resources and a continuity of effort and direction. We already have in the United Kingdom a particular expertise and experience in the mounting and analysis of such long term studies[13]—to cite the most obvious examples: those of Douglas,[14] the two perinatal mortality surveys,[15] and the definitive follow up into adult life of 100 epileptic children by Ounstead *et al.*[16] Other examples are those of the MRC on the outcome of phenylketonuria[17] and neonatal hypothyroidism,[18] those in babies conceived by in vitro fertilisation, the Oxford Neonatal Epidemiology Unit, and Dr Jane Somerville's follow up studies on children operated on for congenital heart disease.[19] It is a sad fact, however, that many of these enterprises are likely to fold up for lack of long term commitment before their potential has been full realised,[20] and for the same reason, other desirable studies in parallel fields will never be started. What is more, many data 'banks' are never fully analysed and sometimes may not be adequate for answering the most obvious questions—for example, the first perinatal mortality survey did not record the birth status of the cohort of the babies identified and followed up, with the result that we do not have the answer to Dr Murray's question that could by now have been forthcoming.

The MRC has—in the opinion of some—to an extent neglected the brief that its title implies by concentrating on work that is at a given time fashionable, or attractive for its so called 'elegance', rather than on fulfilling its long term responsibility as a source of valid information on the causes and cure of diseases and their prevention in our population. Yet why else does it distribute its funds in the support of both 'projects' (short term) and 'units' (long term). Is there not a case for the establishment of a *very* long term unit whose direction would not depend on one man or woman but could be renewed every decade or so in order to feed new ideas, impetus, and enthusiasm into work that could otherwise degenerate into what Bacon called the science of the ant: 'they only amass and store'. Such a unit would have to be staffed by dedicated, even obsessional, workers who were willing to wait for decades for their questions to be answered and to store up information as a future resource for their academic heirs. Fortunately, the advent of the computer has made such labour much less tedious and unmanageable than it used to be. Refrigeration allows for the long term storage of specimens whose future analysis (like the blood on the weapons of our stone age ancestors!) may provide information that those who collected them would not have foreseen as being relevant or obtainable.

What is envisaged is a unit with its own premises, staff, and facilities under the direction of an epidemiologist with the drive and imagination to collect national data in large cohorts of babies and children and adolescents. The help of genetic, obstetric, clinical biochemistry, and paediatric departments would be needed in order to follow the progress and determine the prognosis of the large variety of identifiable or definable disorders or illnesses that may turn out to have long term sequelae, and which could be used to test retrospective hypothesis or predictions based on theoretical considerations. Such a unit could for a start have referred to it by the MRC the follow up of all major screening and immunisation programmes. If the MRC cannot take a long view at a national level, who else can be expected to do so? If no one does, will we not be condemned in the future, if we survive as a culture and society, as having had no long term vision in a field that above all now requires it?

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References

[1] Barker DJP. Childhood causes of adult illness. *Arch Dis Child* 1988;**63**:867–9.

[2] Barker DJP, Osmond C. Childhood respiratory infection and adult chronic bronchitis in England and Wales. *Br Med J* 1986;**293**:1271–5.

[3] Black D, Morris JN, Smith C, Townsend P. *Inequalities and health*. London: DHSS, 1980. (Black report.)

[4] Nicholls KM, Fairley KF, Dowling JP, Kincaid-Smith P. The clinical course of mesangial IgA associated nephropathy in adults. *Q J Med* 1984;**53**:227–50.

[5] Kass H, Savage W, Santamarina BA. The significance of bacteriuria in preventive medicine. In: Kass EH, ed. *Progress in pyelonephritis*. Philadelphia: FA Davis, 1965:3–10.

[6] Smellie JM, Prescod N. Natural history of overy urinary infec-

tion in childhood. In: Asscher AW, Brumfitt W, eds. Microbial diseases in nephrology. Chichester: John Wiley, 1986:241–55.

[7] Jacobson SH, Eklöf O, Eriksson CG, Lins LE, Tidgren BO, Winberg J. Development of hypertension and uraemia after pyelonephritis in childhood—27 year follow up. *Br Med J* 1989;**299**:703–6.

[8] Medical Research Council. *Social conditions and acute rheumatism.* London: MRC, 1927. (Child Life Investigations Special Report Series No 114.)

[9] Barker DJP, Osmond C, Golding J, Kuh D, Wadsworth MEJ. Growth in utero, blood pressure in childhood and adult life, and mortality from cardio vascular disease. *Br Med J* 1989;**298**: 564–7.

[10] Whincup PH, Cook DG, Shaper AG. Family influences on blood pressure: a study of children aged 5–7 years. *Br Med J* 1989;**299**:587–9.

[11] Murray RM, Lewis SW. Is schizophrenia a neurodevelopmental disorder? *Br Med J* 1987;**295**:681–2.

[12] Advisory Board for the Research Councils. *A strategy for the science base.* London: HMSO, 1987.

[13] Carpenter L, Beral V, Strachan D, Ebi-Kryston-Kristie L, Inskip H. Respiratory symptoms as predictors of 27 year mortality in a representative sample of British adults. *Br Med J* 1989;**299**:357–8.

[14] Douglas JWB. National survey of health and development. National Child Development Study 1958 Cohort.

[15] Davie R, Butler N, Goldstein H, eds. From birth to Seven: the second report of the National Child Development Study 1958 Cohort. London: Longmans, 1972.

[16] Ounstead C, Lindsay J, Richards P. *Temporal lobe epilepsy 1948–1986; a biographical study.* Oxford: Blackwell Scientific (MacKeith Press). (Clinics in developmental medicine No 103.)

[17] Smith I, Drogari E, Beasley M, Lloyd JK. Timing of strict diet in relation to fetal damage in maternal phenylketonuria. An international collaborative study by the MRC/DHSS phenylketonuria register. *Lancet* 1987;**ii**:927–30.

[18] Grant DB, Smith I. Survey of neonatal screening for primary hypothyroidism in England, Wales and Northern Ireland 1982–4. *Br Med J* 1988;**296**:1355–8.

[19] Somerville J. Congenital heart disease in the adolescent. *Arch Dis Child* 1989;**64**:771–3.

[20] Smith I, Beasley M. Intelligence and behaviour in children with early treated phenylketonuria. A report from the MRC/DHSS phenylketonuria register. *European Journal of Clinical Nutrition* 1989;**43**:1–5.

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Neonatology—then and now *(CHM Walker)*

Anaemia of prematurity (1962)

The prevention of anaemia of prematurity

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St Mary's Hospital for Women and Children, Manchester (Arch Dis Child 1962;**37**:297–9)

It was most unfortunate that, because of the minimal risk of producing a tumour at the site of injection, the use of intramuscular iron-dextran complex, used for some time as an effective prophylactic against late anaemia of prematurity, had to be discontinued. A single injection had a long term effect and avoided the need for daily oral iron. Thus it became necessary to determine if oral treatment could substitute adequately and the conclusions of the work reported in this paper were:

> 'A comparison of mean haemoglobin levels at four week intervals between 57 premature babies who received intramuscular injections of an iron-dextran complex in the newborn period, 75 who received oral iron during the first six months of life and 70 who did not receive any prophylactic iron indicates that the intramuscular preparation was the most effective in preventing the anaemia of prematurity, especially in babies of lowest birth weight. It is suggested that oral iron might be satisfactory if its regular administration could be assured'.

It is also suggested that the introduction of mixed feeding in the fourth month of life is required to maintain a satisfactory haemoglobin level once this has been achieved by the administration of additional iron, but will do no more than check the rate of fall of haemoglobin if additional iron is not given.

Today. While officially recommended doses of oral iron have now been established absorption and utilisation rates vary and compliance is always a problem after the baby goes home. So an awareness that anaemia can 'creep up on one' must be maintained at all times.

There have been several important innovations in treatment since this paper was published. These include the early use of folic acid, vitamin E, and possibly other trace elements but still late anaemia occurs and top up transfusions are required. The part played by iatrogenic loss has been well documented and the answer to the problem would seem to lie in not only minimising blood loss and supplying the substrates but in stimulating the marrow to use them. The role of erythropoietin in this respect is of obvious importance. It is not surprising that the erythropoietin concentration in preterm babies falls in the first few weeks as the premature kidney is unlikely to be able to produce sufficient to maintain adequate concentrations of haemoglobin. As with folic acid, maternal 'donations' are exhaused before the babies can produce enough of their own. One is reminded of work done in the early 1960s by Dr Sandy Spiers of Stirling who stored the plasma taken from blood removed during exchange transfusion which, when given later to the same baby, produced a good red blood cell response. Whether this was due to the erythropoietin, to folic acid content, to both (or to something else) is uncertain but it seems more effective in marrow stimulation than adult donor blood, which seems to have the effect of reducing marrow response to anaemia.

The latest news on erythropoietin is encouraging however. It is now produced by recombinant DNA technology and is available in good quantities.[1] This is not only effective in various forms of anaemia but it has the added bonus, which could be most valuable in preterm babies, in that it also stimulates the production of platelets. Perhaps this at least in part is the long awaited answer to the early marrow inadequacy of the preterm infant.

The principal innovation in assessing the need for and volume of blood transfusion has been the measurement of oxygen carrying power (red cell mass) rather than merely measuring haemoglobin or haematocrit.[2] While not denying its value this technology is not always available and many parts of the world will still have to rely on haemoglobin, and perhaps reticulocytes, to know how the bone marrow is performing and how much blood to transfuse.

References

[1] Cotes, PM. Erythropoietin: the developing story. *Br Med J* 1988;**296**:805–6.

[2] Phillips HM, Holland BM, Abdel-Moiz A, *et al.* Determination of red cell mass in assessment and management of anaemia in babies needing blood transfusion. *Lancet* 1986;**i**:882–4.

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Staff changes

We thank Professor R D G Milner who has retired from his post as associate editor for his critical and constructive work. We thank Professors A G M Campbell and L Spitz who have completed their periods of office as members of the editorial committee, for their conscientious and meticulous work for the journal. We welcome the following as new members of the editorial committee. Professor F Cockburn, professor of child health at the Royal Hospital for Sick Children, Glasgow; Professor E Garralda, professor of child and adolescent psychiatry, St Mary's Hospital, London; Dr H L Halliday, consultant paediatrician with a special interest in neonatology in Belfast; and Mr P Raine, consultant paediatric surgeon at the Royal Hospital for Sick Children, Glasgow. Dr Derek Johnston, who has been a member of the editorial committee for three years, has been appointed associate editor for endocrinology. Thirteen years ago he was appointed consultant paediatrician and senior lecturer in Nottingham. His extensive research has been centred on diabetes mellitus and he has written a popular textbook for undergraduates on paediatrics.

Archives of Disease in Childhood, 1989, **64,** 1758–1759

British Paediatric Association

Minutes of the Annual General Meeting

Minutes of the Annual General Meeting of the Association held on Wednesday 12 April 1989 at the University of York. The President, Professor June Lloyd, took the chair.

1 OBITUARY

The President reported with regret that the following members of the Association had died since the last Annual General Meeting: Professor S A E Ali Taha (Honorary Member); Professor P Bray (Honorary Member); Dr P Congdon; Professor W F Gaisford (Honorary Member and Past President); Dr A G Hesling; Dr D Morris (Honorary Member); Dr Margaret Ounsted; Dr D M Ricks; Dr I D Riley; Professor C A Smith (Honorary Member); Dr B D R Wilson. Members of the Association stood in tribute.

2 MINUTES OF THE 1988 ANNUAL GENERAL MEETING

The minutes of the meeting held on 13 April 1988, which had been circulated to members in March in the Annual Report, were accepted and signed as a correct record.

3 ELECTION OF HONORARY OFFICERS

The meeting ratified Council's election of Dr R MacFaul to serve as Honorary Secretary 1989–92, Dr K L Dodd to serve as Honorary Assistant Secretary 1989–92, Dr Marion Miles to serve as Honorary Assistant Secretary (re-election) 1989–90, and Dr Janet Eyre to serve as Honorary Assistant Secretary (Secretary to the Academic Board) 1989–92.

The President thanked Dr T L Chambers and Professor R W I Cooke for their work as Honorary Secretary and Secretary to the Academic Board over the past five years. Her remarks were endorsed with acclaim.

4 ELECTION OF NEW MEMBERS

Dr B M Ansell, Professor A Ballabriga, Professor K Bozkowa, Professor J A Davis, Professor V W M Drury, The Baroness Faithfull, Professor J O Forfar, Professor J Frézal, Professor E Gautier, Dr J C Graham, Dr L Hanson, Professor R W Smithells, Professor L B Strang, Sir Eric Stroud, and Dr S D W Weller had been proposed for Honorary Membership and there were nominations for 80 Ordinary Members, 10 Overseas Members, and 33 Associate Members. The meeting approved the nominations and the new members were duly elected.

5 HONORARY TREASURER'S REPORT

The Treasurer presented the accounts for the period 1 January to 31 December 1988. These had been circulated to members with the Annual Report and had subsquently been audited. There had been a surplus for the year and the Association's reserves had been increased, largely by substantial profits from sales of the *Archives of Disease in Childhood.*

There had been a regrading of the staff salary structure during the year. The Association's financial status was sufficiently sound to allow plans to be made for the future.

The Honorary Treasurer thanked Mr Michael Ray, the Association's financial adviser, and the secretariat for their work in preparing the accounts.

The Honorary Treasurer's verbal report and the circulated accounts for 1988 were approved.

6 APPOINTMENT OF AUDITORS

The meeting appointed Messrs Arthur G Mead & Co as auditors for the financial year 1 January to 31 December 1989.

7 MEMBERS' SUBSCRIPTIONS

The meeting agreed that the annual subscription rate for Ordinary Members should be increased to a point within the range £115 to £125 from 1 January 1990, with pro rata increases for lesser rates. The final figure would be submitted to Council for approval in June.

8 ANNUAL REPORT

The meeting received the following which had been circulated in the Annual Report to members in March: the report of Council 1988–89; the 23rd Annual Report of the Academic Board; the annual reports of paediatric specialty Associations, Groups and Societies, Standing Committees and Joint Standing Committees, Working Parties, BPA representatives on outside bodies, and paediatricians serving on national bodies. The President thanked all those who had served for their valuable contribution to the aims of the Association, and the office staff for all their hard work.

The President reported that during its Diamond Jubilee year, the Association's profile had been raised and the importance of children's services had been emphasised throughout the country. She drew members' attention to three recent BPA publications: *The History of the British Paediatric Association 1928–1988, Health for All Children,* and the manual on *Infections and Immunizations in Children*.

The President reported that the Joint Planning Advisory Committee had agreed, in the light of the findings of the BPA manpower survey, that there should be an increase in the number of paediatric senior registrars. She thanked members for their cooperation in the survey and gave notice that more work would be undertaken to support further attempts to improve manpower provision.

The President also reported that the Conference of Colleges and Faculties and the BMA wished the medical profession to present a unified response, through the Joint Consultants Committee (JCC), to the principles of the government's White Paper. She would represent the Association at a JCC meeting to be held shortly to finalise this response. As far as services specifically for children were concerned, the BPA Council was preparing a statement on a recommended core service to be provided in each health district. Comments from members were welcome and the final document would be distributed widely.

9 THE FUTURE REPRESENTATION OF PAEDIATRICS

Following the last Annual General Meeting proposals for a College of Paediatricians interdependent with the Royal Colleges of Physicians had been drawn up and a draft charter had been circulated for discussion. The proposals were not supported by the Royal Colleges of Physicians. A paper had been circulated with the March Newsletter reporting on the last meeting of Council, when the views of paediatricians gathered by the regionally elected members of Council had been discussed in the light of the Colleges' response. There was a fairly equal division of opinion among members of the Association between an independent college and a joint Faculty, with a newly emerging strong voice for the development of the status quo. Senior registrars appeared to be more in favour of a separate college. Members had also received with the March Newsletter copies of an initiative formulated by Professor Cyril Chantler which would retain the BPA with enhanced authority and achieve the objectives agreed at the 1987 Annual General Meeting.

After full discussion the following resolutions were carried by a large majority: (a) the Officers should be empowered to negotiate with the Royal Colleges ways in which the objectives previously agreed could be achieved, using as a basis Professor Chantler's proposals and the other suggestions put forward by Council; (b) the results of such negotiations should be reconsidered by Council; (c) the proposals finally agreed by the Colleges and the Council should be submitted to the membership who would decide by referendum whether they were acceptable; (d) the establishment of a Joint Faculty should not be further considered unless the structure of Colleges changed to include Faculties of internal medicine as well as paediatrics.

Archives of Disease in Childhood, 1989, **64**, 1760–1762

Correspondence

Management of asthma: a consensus statement

Sir,

I am writing in response to the special report by Warner *et al.*[1] Basically, I am in agreement with much of what is written in the article and, indeed, it reflects almost verbatim what I have said many times in lectures and written in various articles—with one major exception. The report comes down very heavily against the use of theophylline preparations and is very one sided in its approach. Although I and my colleagues were the authors of one of the articles pointing out the possible behavioural side effects, these were only in a proportion of children in the lower IQ range and we frequently use theophylline preparations without any trouble. This, of course, is also common practice in many other parts of the world. It must also be pointed out that theophylline preparations are far cheaper than cromoglycate and this is a major factor for many patients. Indeed, the report fails to consider the problem of treating asthma in the developing countries where expensive drugs, such as inhaled steroids and cromoglycate, are often unavailable. Finally, the report treats the wheezy infant under 1 year of age as if it were established fact that all the usual antiasthma medications were effective in this age group—a fact which has certainly not been established. In our experience this is the one age when we try to avoid theophylline preparations at all cost because they almost invariably generate severe excitation and contribute little therapeutic effect.

Now I must come to the real problem as I see it. This report was prepared by a group of self appointed albeit very qualified, experts. As such, does publication in the *Archives* constitute formal endorsement of their views by the journal or the British Paediatric Association? Is this policy to be taken, for example, as the standard answer to be expected from candidates being examined for Membership or to be used by lawyers in disputed medicolegal cases? Who funded the meeting and was it entirely free from any commercial pressures or biases? For example, were the manufacturers of theophylline products among the sponsors?

Reference

[1] Warner JO, Götz M, Landau LI, *et al.* Management of asthma: a consensus statement. *Arch Dis Child* 1989;64:1065–79.

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Dr Warner comments:

Thank you for giving me the opportunity to reply to Professor Godfrey's letter. We are sorry that he was unable to attend the meeting in person, having been invited at the same time as all other participants. We have no doubt that his input at the meeting would have been of enormous value. However, I am sure he would agree that the 27 participants from 17 countries have provided a spectrum of informed opinions on the diagnosis and management of childhood asthma.

We believe it goes without saying that any publication in any medical journal does not constitute a formal endorsement of the views expressed in the articles by the journal or the British Paediatric Association. When a large group of well qualified experts actually agree on protocols for the management of a chronic condition, however, this must be considered a reasonably authoritative view. The question of whether the recommendations would ultimately be accepted as the gold standard, either medically or legally, is certainly not in the hands of the participants of the meeting or indeed of the journal that published the article. This will be left entirely to the medical profession at large. However, the deliberations of the Consensus have now been reviewed in a large number of meetings in many countries and have been widely accepted and endorsed. Indeed a precis of the document has been translated into Hungarian and Japanese for distribution to paediatricians. The most recent conference of the European Society of Pneumonology (Freiburg 1989) also had the opportunity to discuss the document at length and considered it to be a significant and important development.

Virtually all meetings, whether organised independantly or as part of a national or international society, are heavily sponsored by drug companies. The Consensus Meeting was also supported by a drug company. However it had absolutely no influence whatsoever over the deliberations and was indeed excluded from participation. Furthermore I would suggest that it is invidious of Professor Godfrey to suggest that the individuals who participated in the construction of this consensus were in any way swayed by commercial pressures or biases.

We are, of course, very pleased that Professor Godfrey is in agreement with most of what is written and indeed it certainly does reflect his multitude of contributions to the medical literature on the management of childhood asthma. It is imperative that we should have a consensus on the management of this condition, which at present is grossly underdiagnosed and undertreated. In 1983 a publication appeared in the *British Medical Journal*, which investigated the specialist approach to childhood asthma. Forty seven specialists answered a questionnaire on the management of childhood asthma. There were considerable differences in opinion for more than half the questions. The authors of this study suggested that 'these results have disturbing implications for the advice that specialists give to general practitioners, children, and parents.' Thus for the sake of all children with asthma, all the participants put in an enormous effort to produce the document. Of course it will require modification in the future and no doubt there will continue to be some disagreement between experts on some of the finer points of management. However, general practitioners and paediatricians without the extra insight of paediatric respiratory physicians do need some basic guidelines to follow. It was also appreciated that there would be some regional differences. As such the Australian paediatric respiratory physicians have already modified the document for local national consumption. They, in fact, have decided to exclude theophyllines completely as being totally unacceptable at any age in the management of asthma and were also unhappy about the use of any other oral bronchodilator. However the remainder of the document was accepted.

The document also clearly outlines that there is virtually no controlled trial evidence of the efficacy of any treatment in children under the age of 1. Thus any judgments on management at this age are best based on clinical experience and anecdotes. It was this age group that generated the greatest discussion in the meeting. However for the remainder, it was accepted even by paediatricians from those countries with a strong inclination to use theophyllines, that they should be demoted to a secondary role in management with the inhaled prophylactic compounds being preferred. When accounting for the costs of the use of treatment, one must also consider the need for monitoring, which of course is essential with theophyllines because of the narrow therapeutic index. However, by comparison there is an enormous margin of safety with the use of the inhaled compounds.

Dr Costain was invited to respond to the letter by Professor Godfrey:

In his letter Professor Godfrey raises several issues which are familiar concerns for those involved in organising consensus conferences and statements. The King's Fund Centre has been organising national concensus conferences since 1984 and has now published statements covering a wide range of issues. It has never been the intention, either explicitly or implicitly, for these to be prescriptive nor had it ever been suggested that the recommendations reflect the only approach to a particular issue. Rather they are statements of what appears to be the best approach given the current state of knowledge. As far as I am aware this is true for consensus statements produced elsewhere and where such statements suggest clinical protocols, they have usually explicitly allowed for deviations to the recommendations to be made where appropriate by clinicians.

On the question of bias, it is not only commercial interests which one has to guard against. We always take considerable care that all interested opinions are considered, although the panel itself clearly has the responsibility for deciding the final recommendations.

There is wide support, both in the United Kingdom and abroad, for the consensus approach to complex or controversial issues. It would be regrettable if fear of misuse were to make production of such statements more difficult.

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Prognostic value of creatine kinase BB-isoenzyme in high risk newborn infants

Sir,

We read with interest the report by Ruth on the prognostic value of creatine kinase BB-isoenzyme (CK-BB) in the newborn infant.[1] We have studied 14 infants with birth

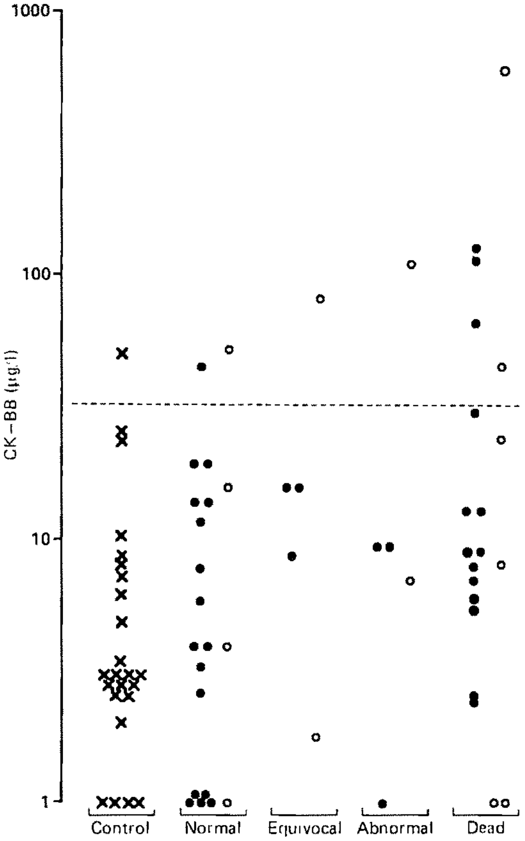

Figure *Serum CK-BB concentrations in asphyxiated (open circles) and very low birthweight (closed circles) infants categorised by outcome, compared with 24 term control infants (crosses). The upper 95% confidence interval derived from the control group is shown.*

asphyxia (those with an Apgar score at 5 minutes <6 or signs of hypoxic ischaemic encephalopathy, or both), 37 very low birthweight infants (<1500 g), and 24 control infants and measured their serum CK-BB concentrations in the first day of life by a two site radioimmunoassay.[2] Neurodevelopmental follow up, including a Griffiths's developmental assessment, was performed at 12–24 months' corrected age. Outcome was graded as 'abnormal' (developmental quotient <75 or definite neurological abnormalities), 'equivocal' (developmental quotient 75–85 or soft neurological signs), or 'normal'. Fourteen very low birthweight and six asphyxiated infants died. In the very low birthweight infants the concentrations of CK-BB in those who died (geometric mean 13·3 µg/l, 95% confidence interval 6·7 to 26·3 µg/l) were significantly higher than those who survived (geometric mean 4·5 µg/l, 95% confidence interval 2·4 to 8·3 µg/l), $p<0{\cdot}05$. If only survivors were considered there was little discrimination of outcome in very low birthweight or asphyxiated babies. The upper 95% confidence interval for the control infants was 32 µg/l, and if this is taken as the upper limit of normal then there was no non-random association between abnormal CK-BB concentration and outcome (figure).

We would concur with Ruth that high concentrations of CK-BB on the first day of life may be predictive of death but are not a useful indicator of outcome in survivors.

References

[1] Ruth VJ. Prognostic value of creatine kinase BB-isoenzyme in high risk newborn infants. *Arch Dis Child* 1989;**64**:563–8.

[2] Jackson AP, Siddle K, Thompson RJ. Two-site monoclonal antibody assays for human heart- and brain- type creatine kinase. *Clin Chem* 1984;**30**:1157–62.

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  BROOK C G D, *editor*: Clinical paediatric endocrin logy. 2nd ed. 1989. **1520**

  BRUTON C J The neuropathology of temporal lo epilepsy 1988. **434**

  BUCHANAN N Child and adolescent health for prac tioners 1988. **312**

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  COOK D and MARCY J, *editors*: Neonatal anesthe 1988. **512** *fn*

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  CREASY R and RESNIK R, *editors*: Maternal fet medicine. Principles and practice 1989. **1524**

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HANSON L A, *editor*: Biology of human milk, (Nestlé Nutrition Workshop Series Vol 15.) 1988. **642**
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HILL A and VOLPE J, *editors*: Fetal neurology 1989. **1098**
JAMES D and STIRRAT G Pregnancy and risk 1988. **1099**
JELLIFFE D B and JELLIFFE E F P, *editors*: Programmes to promote breastfeeding 1988. **640**
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LAIDLAW J, RICHENS A and OXLEY J, *editors*: A textbook of epilepsy 1988. **434**
LARAYA-CUASAY L R and HUGHES W T, *editors*: Interstitial lung diseases in children. Vol I, II, and III 1988. **1521**
LEVENE M, BENNETT M and PUNT J, *editors*: Fetal and neonatal and neurosurgery 1988. **511** *fn*
LUTEN R C, *editor*: Problems in pediatric emergency medicine 1988. **1522**
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MILLA P J, *editor*: Disorders of gastrointestinal motility in childhood 1988. **432**
MODELL M and BOYD R Paediatric problems in general practice, 2nd ed. 1988. **1098**
MORRAY J P Pediatric intensive care 1987. **310**
MULLIKEN J B and YOUNG A E Vascular birthmarks – hemangiomas and malformations 1988. **1524**
NICOLL A and RUDD P, *editors*: Manual on infections and immunizations in children 1989. **1518**
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OPITZ J M, *editor*: Topics in pediatric genetic pathology 1987. **437**
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SCHWARTZ P J, SOUTHALL D P and VALDES-DAPENA M, *editors*: The sudden infant death syndrome. Cardiac and respiratory mechanisms and interventions 1988. **1522**
SIEGEL M J, *editor*: CICT: pediatric body CT. Vol 10 1988. **432**
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The 12th Meeting of the European Society for Paediatric Haematology and Immunology (ESPHI) held in Paris, 19–21 July 1989 **685**

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F. Ginsberg-Fellner, New York, NY;
R. C. McEvoy, City University of New York, NY (Eds.)

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D. Schlossberg, Temple University, Philadelphia, PA (Ed.)

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F. Sharp, R. B. Fraser, R. D. G. Milner, Sheffield (Eds.)

Fetal Growth

1989. VIII, 428 pp. 71 figs. Hardcover
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J. S. E. Moyes, V. R. McCready, A. C. Fullbrook, Sutton

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B. Lachmann, University of Rotterdam (Ed.)

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1990. Approx. 464 pp. 70 figs. 95 tabs.
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J. Yokoyama, Keio University, Tokyo;
T. A. Angerpointner, Munich (Eds.)

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